The Pathogenesis And Treatment Of Severe Acute Pancreatitis

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Doctor of Medicine (MD)

The University of Edinburgh

2001

Declaration

I affirm that:

(a) The thesis has been composed by myself.

(b) The work forming the thesis is my own.

(c) The work forming the basis for the thesis was undertaken solely in South-East Scotland.

(d) The thesis has not been submitted for any other degree, diploma or professional qualification.

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Abstract

Current models hold that organ dysfunction in acute pancreatitis is mediated through a systemic inflammatory response characterised by the secretion of pro-inflammatory mediators and the activation of leukocytes and their subsequent interaction with activated endothelial cells. Although pancreatic inflammation is the initial trigger for the systemic inflammatory response, recent evidence suggests that it is maintained in part by intestinal tract dysfunction, which in turn is partly mediated by the loss of enteral nutrition. The aim of this thesis is to investigate the basis for disease severity in acute pancreatitis, and to assess a novel therapeutic intervention in patients with severe acute pancreatitis.

1) To provide evidence for the presence of endothelial cell activation the kinetics of serum soluble E-selectin and P-selectin in 18 patients with acute pancreatitis were determined. In all patients soluble P-selectin concentrations decreased significantly over the study period. Non-survivors had significantly higher levels of soluble P-selectin than survivors. In contrast, soluble E-selectin increased significantly over the study period in patients with organ dysfunction, whilst remaining constant in patients without evidence of organ dysfunction.
2) Cytokines such as TNFα and IL1β and their endogenous antagonists such as IL1RA are important mediators of disease severity in acute pancreatitis. Because secretion of these cytokines is partly determined by genetic factors the distribution of TNF-308, TNFB, IL1β *Taq1* and IL1RN gene polymorphisms were determined for 190 individuals with acute pancreatitis. The frequency of the polymorphisms studied were similar in patients with mild or severe acute pancreatitis. Moreover there was no significant difference in genotype frequency in patients with acute pancreatitis when compared to 102 healthy controls.
3) To further assess the influence of genetic factors, cytokine phenotype for TNFα, IL1β and IL1RA was determined in 51 individuals following recovery. With respect to phenotype, secretion of TNFα was similar in patients with previous mild or severe acute pancreatitis, however the IL1β:IL1RA ratio was

significantly lower in patients with previous severe acute pancreatitis than those with mild disease.

4) To assess the potential benefits from the introduction of enteral nutrition in patients with severe acute pancreatitis a randomised clinical trial was undertaken. Patients were randomised to receive either enteral nutrition or conventional therapy consisting of a nil-by-mouth regime. Of 27 patients, 13 patients received enteral nutrition. A median of 21% of calorific requirements were delivered over the first 4 days by enteral nutrition. There were no significant complications of enteral nutrition. The introduction of enteral nutrition did not significantly affect the concentrations of serum IL6, serum sTNFR-I or serum CRP over the first 4 days of the study period. Although there was no significant differences in intestinal permeability between the two patient groups at admission, by day 4 abnormal intestinal permeability occurred more frequently in patients receiving enteral nutrition.

The present studies therefore suggest:

1) A role for endothelial-derived selectins in the development of organ dysfunction in patients with acute pancreatitis. Further, the observed temporal differences in serum selectin concentrations is in keeping with *in-vitro* observations of endothelial selectin expression.
2) That genetic factors may not be important in determining TNFα secretion in patients with acute pancreatitis. However, a pre-determined imbalance between IL1β and its antagonist IL1RA would appear to exist in patients with severe acute pancreatitis, although the genetic basis for this altered relationship could not be determined.
3) That early enteral nutrition does not ameliorate the inflammatory response in patients with prognostically severe acute pancreatitis. Furthermore early enteral nutrition did not have a beneficial effect on intestinal permeability.

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Acknowledgements

I would like to thank the following individuals:
Mr A.K. Siriwardena for his supervision and guidance throughout my period of research. Also for his advice and providing the original idea behind the enteral nutrition study.
Dr J.A. Ross for his help and supervision of the laboratory aspects of this thesis, in particular the genotype and phenotype studies.
Professor K.C.H. Fearon for his advice on all aspects of the thesis.
Professor D.C. Carter and Professor O.J. Garden for enabling me to carry out my research within the University Department of Surgery, including when necessary financial support.
Mrs J. Maingay and Miss K. Sangster for their technical support.
Mr G. Hill and Mrs S. Storey for their help in running the enteral nutrition trial and collecting blood samples.
Dr J.T. Murchison who placed the nasojejunal feeding tubes.
Dr F.S. di Giovine and Dr A.G. Wilson for their advice and help regarding the study of gene polymorphisms.
Professor G.D. Murray for his advice regarding the study power calculation for the enteral nutrition trial.

I would like to thank the South East Scotland Blood Transfusion Service for their help in recruiting a control population for the genotype studies.

I would also like to thank the University of Edinburgh Faculty of Medicine who provided financial support through the award of an Allan Research Fellowship.

Finally and most importantly, I would like to thank my wife, Jennifer, and the rest of my family who have occasionally suffered in my pursuit of an MD thesis.

Introduction

Acute pancreatitis is an acute inflammatory process of the pancreas, with variable involvement of other regional or remote organ systems [1]. It is a common condition whose incidence appears to be increasing [2]. Although the majority of patients with acute pancreatitis have a mild illness with complete recovery, a substantial proportion of patients develop severe acute pancreatitis. Those patients with severe disease have high morbidity and mortality rates [3], with prolonged in-hospital stays and as a consequence have significant healthcare economic implications [4].

Despite extensive research into the pathogenic mechanisms of acute pancreatitis it is still not clear why a group of patients develop severe disease. Moreover, there are few specific therapeutic interventions for the treatment of acute pancreatitis, and the small improvement in outcome observed over the last couple of decades for patients with severe acute pancreatitis is probably mainly due to improvements in critical care medicine.

With this background in mind the aim of this thesis is to investigate the basis for disease severity in acute pancreatitis, and to assess a novel therapeutic intervention in patients with severe acute pancreatitis.

Demographics Of Acute Pancreatitis

The most recent analysis of Scottish Morbidity Record (SMR) data collected by the Information and Statistics Division (ISD) demonstrated that the incidence of acute pancreatitis in Scotland in 1995 was 41.9 cases per 100,000 population per year [2]. This rate is similar to the incidence of acute pancreatitis in Sweden (38.2 per 100,000/year) [5] and in Norway (41.5 per 100,000/year) [6], but is considerably less than in Finland (73.4 per 100,000/year) [7]. The reason for the difference in the incidence of acute pancreatitis between Scotland and Finland is not clear, but may be due to variation in the prevalence of aetiological agents within each population; the high rate of acute pancreatitis in Finland is associated with a high rate of alcohol consumption within the male population [7].

However, of greater public health concern is the observation that the incidence of acute pancreatitis appears to be increasing. In Scotland during the period 1961 to 1985, a rise from 13.0 to 22.5 cases per 100,000/year occurred [8], although during this period the Phadebas test for the measurement of serum amylase was introduced making the diagnosis of acute pancreatitis easier. However, a further increase to 41.9 per 100,000/year in 1995 has subsequently been observed [2]. Significantly, similar studies from Bristol [9, 10], Nottingham [11], Birmingham [12], Göttingen Germany [13] and Finland [7] have also demonstrated an increase in the incidence of acute pancreatitis. The reason for the observed increase in incidence within each populations is not clear, although in Finland the increase in the incidence of acute pancreatitis is strongly correlated with an increase in alcohol consumption [7].

Aetiology Of Acute Pancreatitis

A number of agents can cause acute pancreatitis (Table 1), although the two most common causes are gallstones and excessive alcohol ingestion; in the United Kingdom gallstones are the most common agent inducing acute pancreatitis. The remaining aetiological agents account for relatively few cases of acute pancreatitis. Further, in spite of extensive investigation the causative agent is frequently not identified. These cases are therefore termed idiopathic acute pancreatitis. It is probable that the number of cases labelled as idiopathic is inversely related to the degree of investigation. Recent United Kingdom guidelines state that the diagnosis of idiopathic acute pancreatitis should account for less than 25% of cases [14].

Common	Uncommon
Gallstones	Trauma
	ERCP
	Sphincterotomy
	Biliary manometry
Alcohol	Pancreatic duct obstruction
	Ampulla of Vater neoplasia
	Drugs
	Azathioprine
	Metabolic
	Hypercalcaemia
	Hyperlipidaemia
	Infection
	Mumps
	Coxsackie B
	HIV
	Vascular
	Vasculitis
	Cardiopulmonary bypass
	Hereditary pancreatitis

Table 1: Aetiological agents in acute pancreatitis.

Pathogenesis Of Acute Pancreatitis

Despite extensive research the pathogenic mechanisms in acute pancreatitis have not yet been fully elucidated. Although a number of disparate agents may induce acute pancreatitis, it is believed that following the initial pancreatic acinar cell injury the pathogenic mechanisms in acute pancreatitis are the same regardless of aetiology. That is, the pathogenic mechanisms giving rise to organ dysfunction in patients with gallstone-induced severe acute pancreatitis are the same mechanisms giving rise to organ dysfunction in alcohol-induced severe acute pancreatitis. Likewise, it is thought that the same basic pathogenic mechanisms underpin both mild and severe acute pancreatitis and that the difference in the clinical picture and outcome is the result of quantitative differences within the pathogenic mechanisms. The reasons for the quantitative differences in the pathogenic mechanisms are not known.

Early Acute Pancreatitis – The Initial Pancreatic Acinar Cell Injury

Various hypotheses have been proposed by which each aetiological agent causes the initial pancreatic acinar cell injury.

Opie initially proposed that gallstones triggered acute pancreatitis in patients in whom the common bile duct formed a common channel with the main pancreatic duct allowing bile to reflux into the pancreatic duct following impaction of a gallstone at the ampulla of Vater. This has become known as the "common channel" theory, and despite a large volume of evidence against it [15] the theory remains popular. Against the theory is the anatomical observation that any "common channel" would be too short to allow reflux of bile into the pancreatic duct during stone impaction [16]. Further, during the initial stages pancreatic duct pressure would be higher than bile duct pressure promoting instead pancreatico-biliary reflux [17, 18]. Similarly, although perfusion of the pancreatic duct with various solutions, including enterokinase-activated pancreatic juice and taurocholate, induces acute pancreatitis in the experimental situation [19, 20], other studies have suggested that the perfusion of

sterile bile at physiological pressures may not induce acute pancreatitis [21, 22]. It is however probable that increased pancreatic duct pressure is the key factor which results in pancreatic acinar cell injury in gallstone-induced disease, although the mechanism through which it mediates acinar cell injury is not known.

Similarly, the mechanisms through which ethanol induces acute pancreatitis have not been elucidated. The postulated initial pathogenic mechanisms of alcohol-induced pancreatitis can be broken down into three broad areas [23] (Table 2) ; "big duct" hypothesis, "small duct" hypothesis and acinar cell injury. The original "big duct" hypothesis was based on Opie's hypothesis regarding the pathogenesis of gallstone-induced disease, and held that acute pancreatitis was a consequence of biliary obstruction due to alcohol-induced sphincter of Oddi spasm or duodenitis in individuals with a common channel allowing bilio-pancreatic reflux. However, subsequent studies have provided cogent evidence against the original and subsequent variants of the big duct theory [24]. More probable explanations may be provided by the "small duct" and acinar cell injury theories, although it should be noted that neither of these theories is mutually exclusive. Sarles and Sahel [25] postulated that precipitation of protein plugs within pancreatic ductules is an important early event resulting in obstruction. Although precipitation of protein is due in part to increased protein concentration [26], increased pancreatic acinar cell secretion of lactoferrin, an iron containing protein which has the ability to stimulate acidophilic protein aggregation, also occurs [27]. The cause of the abnormal protein secretion is not known.

Other potential mechanisms of alcohol-induced acinar cell injury exist. As in alcoholic liver disease a toxic ethanol metabolite, such as acetaldehyde, may be the cause of the pancreatic acinar cell injury [23]. Although acetaldehyde may be directly toxic to the pancreatic acinar cell, metabolisation of acetaldehyde by xanthine oxidase releases oxygen free radicals which may also mediate pancreatic acinar cell injury [28].

Finally, alcohol-induced acute pancreatitis may be the consequence of abnormal trafficking of proteins within the pancreatic acinar cell, whereby co-localisation of digestive pro-enzymes and lysosomal enzymes occurs with subsequent secretion of activated pancreatic enzymes into the pancreatic parenchyma leading to the induction of acute pancreatitis [24].

Big duct hypotheses
Biliary-pancreatic reflux
Sphincter of Oddi obstruction
Duodenal-pancreatic reflux
Increased ductal permeability
Small duct hypotheses
Increased viscosity-hypersecretion of proteins
Increased lactoferrin
Decreased lithostatine
Acinar cell injury
Toxic metabolites
Unopposed free radical injury
Stimulation of leucocytes
Lysosomal hyperactivity
Cholinergic hyperactivity
Abnormal protein trafficking
Necrosis-fibrosis sequence

Table 2: Potential mechanisms through which alcohol induces acute pancreatitis.

Early Acute Pancreatitis – Abnormal Intracellular Physiology

Although the pathogenic mechanisms through which an initiating stimulus causes pancreatic acinar cell injury may differ, it is believed that a common pathway exists through which abnormal intracellular metabolism leads to the premature activation of pancreatic exocrine pro-enzymes in an inappropriate location.

Experimental studies have suggested that the pro-enzyme trypsinogen is important. Under normal physiological conditions, following secretion of trypsinogen into the

lumen of the small intestine, trypsinogen is cleaved through the action of enterokinase to the active enzyme trypsin. However, within cell culture, intracellular activation of trypsinogen can be provoked in isolated pancreatic acinar cells using stimuli which in animal models may induce acute pancreatitis. Similarly in experimental animal models of acute pancreatitis intracellular activation of trypsinogen can be observed alongside aberrant trypsinogen activation within the pancreatic parenchyma [29, 30]. The cause of the aberrant activation and secretion of trypsinogen is not clear, although a disturbance in normal calcium metabolism has been implicated [31, 32].

Further evidence supporting the role of intracellular activation of trypsinogen in the pathogenesis of acute pancreatitis comes from the identification of mutations predisposing to hereditary acute pancreatitis within the cationic trypsinogen gene. Under normal conditions auto-activation of cationic trypsinogen occurs at low levels, however a number of protective mechanisms exist to prevent cellular damage. In the first instance, an intracellular peptide termed pancreatic secretory trypsin inhibitor (PSTI) exists which irreversibly binds to trypsin thereby inactivating it [33]. However, if levels of intracellular trypsin exceed the capacity of pancreatic secretory trypsin inhibitor, trypsin itself activates intracellular proteases which in turn cleave the activated trypsin thereby neutralising it. In hereditary pancreatitis a mutation occurs within the cationic trypsinogen gene rendering cationic trypsin resistant to the action of intracellular proteases [34], negating the effect of the negative feedback mechanism.

Acute Pancreatitis – Local Pancreatic Inflammation

The effect of premature activation of intracellular and intra-parenchymal trypsinogen is the induction of local pancreatic inflammation.

In the early stages of acute pancreatitis leukocytes accumulate within the pancreas, their numbers correlating with the severity of the acute pancreatitis [35, 36], and pancreatic capillaries demonstrate increased permeability [37, 38]. The pathogenesis of

the local acute inflammatory response appears similar to that at any other site. A large number of interacting mechanisms and mediators have been implicated in local pancreatic inflammation (Table 3). Moreover, experimental studies in animal models have demonstrated reduced pancreatic inflammation following interventions aimed at perturbing these mechanisms. Although the inflammatory response is likely to be triggered by the aberrant secretion of pancreatic enzymes into the pancreatic parenchyma other mechanisms may also play a role. Pancreatic acinar cells have the ability to express pro-inflammatory cytokines and chemokines following an insult [39,40]. Further, once initiated, the inflammatory process may become independent of further protease activation [36], that is the local inflammatory response may become self sustaining.

Proteases (e.g. trypsin)
Cytokines (e.g. TNFα, IL1β, IL1RA)
Chemokines (e.g. IL8)
Platelet activating factor
Arachidonic acid metabolites
Reactive oxygen species
Endothelins
Leukocyte margination
Altered microcirculation
Increased capillary permeability

Table 3: Mediators and mechanisms of local pancreatic inflammation.

Acute Pancreatitis – Systemic Inflammatory Response

Acute pancreatitis has long been recognised as having effects distant from the pancreas gland. It is these remote effects which, in part, distinguish those patients with severe acute pancreatitis from those with mild disease [1]. Original hypotheses held that multiple organ dysfunction in acute pancreatitis arose as a consequence of

circulating activated digestive enzymes causing systemic "autodigestion". However, following the failure of protease inhibitors to ameliorate the disease process [41] and more recent evidence derived from both human and experimental animal models newer models have been proposed. Rinderknecht [42] hypothesised that an excessive systemic inflammatory response is responsible for the development of multiple organ dysfunction, and that the mechanisms responsible for the development of multiple organ dysfunction in acute pancreatitis are fundamentally the same as those found in other critical illness states. Current models therefore hold that organ dysfunction in acute pancreatitis is the consequence of a systemic inflammatory response which is characterised by the secretion of inflammatory mediators, the activation of leukocytes and their interaction with endothelial cells [43].

Inflammatory mediators

Tumour necrosis factor

Tumour necrosis factor α (TNFα) and tumour necrosis factor β (TNFβ) are members of the tumour necrosis factor ligand and receptor family. This group of biologically active molecules consists of 10 known ligands with similar biological properties. In general, members of the tumour necrosis factor superfamily play major roles in the pathogenesis of inflammation-mediated diseases.

TNFα was the first described member of the tumour necrosis factor ligand family and was so named because of its ability to cause necrosis within transplantable tumours [44]. However, despite initial expectations that TNFα could be used as a novel anti-cancer agent, subsequent studies in both animals and humans demonstrated highly toxic effects, with TNFα effecting a state akin to septic shock. Indeed, TNFα has subsequently been demonstrated to be a potent pro-inflammatory mediator with a key role in the development of the systemic inflammatory response syndrome and multiple organ failure.

TNFα is a 233 amino acid, non glycosylated polypeptide which exists in both a 26kDa membrane associated form and a 17kDa soluble form [45-47]. The 17kDa soluble form arises from proteolytic cleavage of membrane-associated TNFα through the action of TNFα converting enzyme [48, 49]. Soluble 17kDa TNFα combines non-covalently to form a homotrimeric complex, which spontaneously decays with a half life of 5-10 minutes [50, 51]. Although both the membrane-associated and the soluble homotrimer are biologically active, soluble homotrimeric TNFα is the more potent [52, 53].

Tumour necrosis factor β (TNFβ) or lymphotoxin is a 171 amino acid residue, 25kDa glycosylated polypeptide [54] which has 28% homology with TNFα [45, 55]. In contrast to TNFα, TNFβ does not have a transmembrane form, existing solely as a biologically active soluble homotrimeric form. TNFβ may however be membrane associated through its interaction with membrane associate lymphotoxin β [56, 57]. Although the biological effects of TNFβ are similar to those of TNFα, little is known about its role in human critical illness states because the emphasis of research has been focussed on TNFα.

TNFα is secreted by many different cells, although the principal cellular sources are derived from the monocyte/macrophage cell line. TNFα secretion is stimulated by a number of bacterial, viral, parasitic and tumour related products, as well as other pro-inflammatory mediators including TNFα itself [58]. One of the most potent stimuli is endotoxin, a lipopolysaccharide derived from the outer membrane of gram negative bacteria. TNFα mediates a wide range of responses (Table 4).

Pyrexia

Cardiovascular

Hypotension
Myocardial suppression
Capillary leak syndrome
Lactic acidosis

Pulmonary

Acute respiratory distress syndrome

Haematological

Leukocytosis
Suppression of erythropoiesis
Disseminated intravascular coagulation

Glucose metabolism

Hyperglycaemia
Hypoglycaemia

Mediation of hepatic acute phase protein synthesis

Decreased synthesis – Albumin

Table 4: The effects of TNFα [59].

Evidence derived from both experimental models and clinical studies has demonstrated that TNFα is an important early mediator in the development of the systemic inflammatory response syndrome and multiple organ dysfunction [59]. In experimental animal models the administration of gram-negative bacteria increases the level of circulating serum TNFα, provoking a state similar to septic shock [60]. Moreover the infusion of TNFα alone is sufficient to provoke the septic state [61], whilst antagonism of TNFα through the administration of either anti-TNFα antibodies [62] or recombinant soluble tumour necrosis factor receptors [63] in animal models of sepsis is associated with an improved outcome.

However, although there is compelling evidence derived from animal experimental studies for a major role for TNFα in the development of the systemic inflammatory response and multiple organ dysfunction, data relating to human critical illness states are less strong. Certainly intravenous infusion of endotoxin provokes signs of a systemic inflammatory response and is associated with increased levels of

TNFα [60, 64, 65], whilst the infusion of TNFα alone induces signs of a systemic inflammatory response [66-68]. However, TNFα is infrequently detected in the serum of patients with the systemic inflammatory response syndrome or multiple organ dysfunction [69]. Although the failure to detect TNFα in all patients may suggest that TNFα does not play a role in the pathogenic process, it may also be a manifestation of sampling error brought on by the very early role of TNFα in the development of the inflammatory state coupled with the short half-life of circulating TNFα. Similarly, although several large clinical trials have demonstrated that blockade of TNFα activity does not improve outcome in patients with the systemic inflammatory response syndrome [70-75], there are several potential explanations which may explain these results including the very proximate role of TNFα in the pathogenic process and the overall complexity of the systemic inflammatory response [76].

As may be predicted, the role of TNFα in the pathogenesis of acute pancreatitis would appear to be similar to its role in the mediation of the systemic inflammatory response and multiple organ dysfunction in other critical illness states.

In experimental models of acute pancreatitis, TNFα production occurs initially within the pancreas and then subsequently within other remote organs [39, 77-80], although there does appear to be differential organ synthesis of TNFα with marked expression in lung, liver and spleen but not in kidney, cardiac muscle or skeletal muscle [77].

Further, perturbation of the mechanisms leading to TNFα secretion results in improved outcome in animal models of acute pancreatitis. Inhibition of the p38 MAP kinase intracellular signalling pathway, which is involved in TNFα transcription, ameliorates the acute lung injury in acute pancreatitis [81]. Similarly prevention of NFκB mediated tumour necrosis factor gene transcription reduces the severity of experimental acute pancreatitis [82], whilst inhibition of TNFα mRNA translation reduces the severity of the pancreatic and lung injury [83].

Similarly, evidence demonstrating the importance of TNFα in the pathogenesis of acute pancreatitis has been derived from experimental studies which aim to block the effects of TNFα. Although an initial study which examined the effects of the administration of a polyclonal anti-TNFα antibody in an animal model of acute pancreatitis suggested a worsening of disease severity [84], two further studies using different polyclonal anti-TNFα antibodies have demonstrated improved outcome [85,86]. Moreover, infusion of recombinant soluble TNF receptor I improves outcome [87]. Finally the severity of experimentally induced acute pancreatitis is less in TNF receptor I gene knockout mice when compared to normal wild mice [88].

Again however, the evidence relating to TNFα in human acute pancreatitis is less strong when compared to that derived from animal studies. Even in severe acute pancreatitis, TNFα can only be detected in the serum of a minority of cases [89-93]. The reason for the failure to observe serum TNFα is not clear but again it may be a consequence of the short half life of TNFα coupled with infrequent blood sampling. Other explanations may exist. In an animal model of acute pancreatitis, levels of serum TNFα were significantly higher in portal venous blood compared with systemic venous blood; the lower systemic levels attributable to hepatic metabolism [94]. Therefore undetectable serum levels may not be indicative of a lack of TNFα production but may be the result of the compartmentalisation of cytokine production with the measurement being taken from the "wrong" compartment.

However, in contrast to the infrequent detection of TNFα in the serum of patients with acute pancreatitis, serum soluble TNF receptors can be detected in all cases with levels of serum soluble TNF receptor type I [91, 95, 96] and serum soluble TNF receptor type II [91] correlating with disease severity. Significantly because levels of serum soluble TNF receptors correlate with levels of serum TNFα in a human model of endotoxaemia [97], and infusion of recombinant TNFα provokes a rise in serum soluble TNF receptors [98], serum soluble TNF receptors may be considered as an indirect measure of previous TNFα exposure. The observations of de Beaux [91],

Kaufmann [95] and Soong [96] therefore suggest that there is significant production of TNFα in patients with severe acute pancreatitis.

The cellular origin of increased systemic TNFα production in acute pancreatitis is not clear. Although pancreatic acinar cells can synthesise TNFα [39], significant first pass hepatic metabolism of TNFα occurs [94] and therefore other cellular sources of TNFα are more likely to be involved in the pathogenesis of the systemic inflammatory response. The most probable source is cells derived from the monocyte/macrophage line. McKay and colleagues [99] observed that monocytes isolated from patients with severe acute pancreatitis secrete greater amounts of TNFα than those from patients with mild disease. However, similar differential secretion has not been observed with isolated peripheral blood mononuclear cells, which consists of mixed monocytes and lymphocytes [100].

Two types of tumour necrosis factor receptor have been identified. TNF receptor type I (TNFR-I) is a 55kDa transmembrane protein and is expressed by many cell types including monocytes and neutrophils [101], endothelial cells [102] and hepatocytes [103]. TNF receptor type II (TNFR-II) is a 75kDa transmembrane receptor which is expressed on a variety of cell types including monocytes [104], macrophages [105] and endothelial cells [106]. The relative importance of each type of receptor in mediating intracellular events is not clear. Initial studies suggested that the majority of TNFα effects were mediated through TNFR-I, with TNFR-II receptor acting as a carrier of TNFα to TNFR-I receptors. However, TNFR-II alone may mediate TNFα-induced apoptosis in rhabdomyosarcoma [107] and Langerhans cell migration [108]. Further, it has been suggested that TNFR-II receptors may mediate the effects of membrane associated TNFα [109].

TNFR-I and TNFR-II also occur as soluble forms (sTNFR-I, sTNFR-II). These soluble forms arise from the proteolytic cleavage of the extra-cellular domains of the membrane-associated forms [110]. Although the exact mechanisms stimulating membrane associated receptor cleavage are not clear, because receptor shedding occurs after cell stimulation by TNFα, it has been suggested that receptor shedding

may be a mechanism through which cellular down-regulation can occur. However, other biological effects may arise from the release of soluble TNF receptors. Although soluble TNF receptors can bind free soluble TNFα thereby reducing the bioactivity of TNFα [59, 111, 112], binding may also act to stabilise homotrimeric TNFα delaying its spontaneous decay [113]. Therefore soluble TNF receptors may act as a buffer of TNFα activity either prolonging or neutralising TNFα effects depending on the prevailing TNFα conditions.

However, although the function of soluble TNF receptors is not clear, studies have clearly demonstrated a relationship between the concentration of serum soluble TNF receptors and the inflammatory process. In human experimental models of sepsis the concentration of serum soluble TNF receptors correlates with peak levels of TNFα following exposure to endotoxin [97], whilst infusion of recombinant TNFα leads to an increase in circulating soluble TNF receptors [98]. Serum sTNFR-I and sTNFR-II concentrations are therefore considered to correlate with the degree of previous exposure to TNFα. Similarly, clinical studies have demonstrated an association between serum soluble TNF receptors concentrations and clinical outcome in inflammatory states. In critical illness states, shedding of monocyte membrane-associated TNF receptors is associated with increased disease severity and adverse outcome [114], whilst concentrations of serum sTNFR-I and sTNFR-II correlate with disease severity [69, 115]. Likewise, concentrations of serum soluble TNF receptors correlate with disease severity in acute pancreatitis [91, 95, 96]. Serum soluble TNF receptor concentration may therefore be used as a measure of the inflammatory response in critical illness states including acute pancreatitis.

Interleukin 1

The interleukin 1 family consists of 3 proteins; interleukin 1α (IL1α), interleukin 1β (IL1β) and interleukin 1 antagonist (IL1RA).

IL1α and IL1β are 17kDa proteins with 26% amino acid homology [116]. IL1α and IL1β are secreted primarily by cells of the monocyte/macrophage line although they can be synthesised by most other nucleated cells [117]. Both IL1α and IL1β bind to interleukin 1 receptor type 1 and 2, but their effects are mediated solely through interleukin 1 receptor type 1 (Table 5); interleukin 1 receptor type 2 binds interleukin 1 but does not transduce a signal [118]. However, although their cellular effects are broadly similar, differences in the pattern of expression may influence their biological role. IL1α is not readily secreted from the synthesising cell [119, 120] but is biologically active either as precursor IL1α within the intracellular matrix, or following processing as a membrane-associated protein [121]. In contrast, IL1β exerts its effect following extracellular secretion [122]. These differences suggest that IL1β has a predominately systemic effect whilst IL1α is primarily a regulator of local inflammation and intracellular events.

Pyrexia

Cardiovascular

Hypotension
Myocardial suppression
Lactic acidosis

Glucose metabolism

Hyperinsulinaemia
Hyperglycaemia
Hypoglycaemia

Haematological

Leukocytosis
Thrombocytosis

Mediation of hepatic acute phase protein synthesis

Increased synthesis – C-reactive protein, complement, clotting factors
Decreased synthesis – Albumin

Increased gene expression

Cytokines – TNF, IL1, IL1RA, IL8
Pro-inflammatory mediators – cyclo-oxygenase, endothelin
Clotting factors – fibrinogen, tissue factor
Hepatic acute phase proteins – C-reactive protein, serum amyloid A
Adhesion molecules – L-selectin

Decreased gene expression

Receptors – TNF receptor type 1, IL1 receptor type 1

Table 5: The effects of interleukin 1 [121].

IL1RA is a 22 to 25kDa protein the synthesis of which may be induced in most cells. IL1RA demonstrates homology to IL1α (18%) and IL1β (26%) [123, 124] but in contrast is a pure antagonist of interleukin 1 receptor type 1 and 2 [125]. The majority of active IL1RA is extracellular but through the mechanism of intracellular gene splicing an active intracellular form has been described [122].

A large number of agents can induce the synthesis of interleukin 1 [121] (Table 6) . Considering both the nature of the agents able to induce IL1 secretion as well as the effects of IL1 it would appear that the interleukin 1 family members are important mediators of the inflammatory response. However, because of the potential for the interleukin 1 family members to interact with each other as well as with both membrane-associated and soluble interleukin 1 receptors, predicting their effects is

not straightforward. Both IL1α and IL1β may be antagonised by IL1RA at the receptor level, whilst IL1β may interact with circulating soluble interleukin 1 receptors thereby buffering IL1 activity. This potential complexity may provide an explanation for the observation that whilst improved outcome has been demonstrated in animal models of sepsis following the administration of anti-interleukin 1 agents [126-129] these benefits have not been observed in the setting of a randomised controlled clinical trial [130].

Microbial factors
Bacteria
Yeasts
Viruses
Stress factors
Ischaemia/reperfusion
Hyper-osmolarity
Thermal injury
Cytokines
TNF
IL1
Platelet derived growth factor
Lipids
Platelet activating factor
Inflammatory mediators
Complement
C-reactive protein
α-1 anti-trypsin
Clotting factors
Thrombin
Fibrin degradation products
Plasmin
Cell matrix
Fibronectin
Collagen
Neuro-active substances
Substance P

Table 6: Agents which may induce the synthesis of interleukin 1 [121].

However, regardless of the potential complexity of the system, studies have suggested that the interleukin 1 family has an important role in the mediation of acute pancreatitis.

Up-regulation of IL1β synthesis occurs within the inflamed pancreas mainly within leukocytes [77, 131, 132], and is accompanied by an increase in IL1RA synthesis [132]. Concomitantly, increased IL1β synthesis occurs in remote organs, and parallels the increase in systemic TNFα synthesis [77].

Again, manipulation of the mechanisms through which IL1β is secreted and mediates its effects, results in amelioration of disease severity in experimental models of acute pancreatitis. Inhibition of interleukin 1 converting enzyme, which is required for post-translational processing of IL1β, results in a reduction in disease severity in an experimental model of acute pancreatitis [133]. Similarly, gene knockout of IL1 receptor 1 (IL1R-I) leads to a reduction in severity of experimental acute pancreatitis [88, 134], although levels of IL1β mRNA increase suggesting the involvement of IL1R-I in a negative feedback loop [135]. Finally the administration of IL1RA in experimental models of acute pancreatitis reduces disease severity and mortality [136-138].

These animal experimental studies have also demonstrated that a close interaction occurs between IL1β and TNF α in the mediation of acute pancreatitis. Inhibition of interleukin 1 converting enzyme in acute pancreatitis results in a reduction in levels of serum IL1β but is also associated with a reduction in serum TNFα [133], whilst the survival benefits of combined IL1R-I and TNFR-I gene knockout are greater than IL1R-I or TNFR-I gene knockout in isolation [88].

However, despite the evidence regarding the role of IL1β and IL1RA in the pathogenesis of experimental acute pancreatitis there are few data available in relation to human acute pancreatitis. Brivet and colleagues [89] observed that although levels of serum IL1RA were raised in patients with severe acute pancreatitis, IL1β could only be detected in the serum of 3 of 50 study subjects. Similarly McKay and

colleagues [99] found no difference in IL1β secretion from monocytes isolated from patients during an episode of mild or severe acute pancreatitis.

Interleukin 6

Interleukin 6 (IL6) is a member of the gp130 signalling cytokine family. IL6 is constitutively synthesised in the spleen, liver, kidney and intestinal tract and also by peripheral leukocytes [139]. Synthesis may however be induced in almost every cell type following an appropriate stimulus [139]. The role of IL6 in critical illness states is not entirely clear. Although IL6 is a major mediator of the hepatic acute phase protein response, it has also been demonstrated to have both pro-inflammatory and anti-inflammatory effects [120, 139-142]. However, regardless of its exact role in the pathogenesis of a systemic inflammatory response, IL6 production is correlated closely with disease severity in acute pancreatitis.

A number of studies have reported that the concentration of serum IL6 is significantly higher in patients with severe acute pancreatitis when compared to those with mild disease [91, 143-147], and that IL6 levels precede the hepatic acute phase protein response, as manifested by C-reactive protein concentrations, by 24 to 48 hours [91, 143, 147]. The source of the increased serum IL6 is not clear. In an experimental animal model Norman and colleagues [77] suggested that IL6 synthesis was confined to the pancreas, however McKay and colleagues [99] have reported that monocytes isolated from patients with severe acute pancreatitis demonstrate increased IL6 secretion when compared to patients with mild disease. Similarly de Beaux and colleagues [100] have shown that IL6 production per unit of blood is increased in patients with severe acute pancreatitis.

In summary, although relatively little is known regarding the pathogenic role of IL6 in acute pancreatitis the close correlation between serum IL6 and clinical outcome has resulted in its use as a marker of disease severity.

Interleukin 8

Interleukin 8 is a pro-inflammatory chemokine secreted by a variety of cell types [148]. The major effects of IL8 are upon neutrophils, stimulating chemotaxis, adhesion, superoxide release and bacterial killing. Evidence is accumulating that IL8 plays an important role in inflammatory conditions including acute pancreatitis. In patients with acute pancreatitis serum concentrations of IL8 are significantly higher in patients with severe disease when compared to those patients with mild disease [145, 146, 149, 150]. Moreover serum concentrations of IL8 correlate with the degree of organ dysfunction [151]. The source of the increase in serum IL8 concentrations is not clear, however in patients with severe disease IL8 secretion by monocytes is increased [99], as is the secretion of IL8 per unit of blood [100]. Further supporting the importance of IL8 in mediating the systemic effects of acute pancreatitis is the observation that in an animal model of acute pancreatitis the administration of an anti-IL8 antibody attenuated the cytokine response and reduced acute lung injury but did not reduce the degree of pancreatic inflammation [152].

Platelet Activating Factor

Platelet activating factor (PAF) is a lipid derived pro-inflammatory mediator that is released from cell membrane lipids under the action of phospholipase A2. Studies have suggested a role for PAF in the pathogenesis of acute pancreatitis. In an animal model, the injection of PAF into the arterial blood supply to the pancreas provokes lesions within the pancreas that are morphologically consistent with acute pancreatitis [153]. Further, following the induction of experimental acute pancreatitis, PAF can be detected in both the pancreas and lung [154, 155]. Moreover, the administration of PAF antagonists result in the amelioration of both local pancreatic injury [156-158] and remote organ injury [155, 159, 160]. However, benefits derived from the administration of a PAF antagonist in experimental models are not observed if therapy is delayed until after the induction of acute pancreatitis [161]. Interestingly though, the administration of recombinant PAF-acetyl hydrolase, an enzyme

involved in PAF degradation, after the induction of experimental acute pancreatitis resulted in the amelioration of the acute lung injury [162]. These promising experimental results have led to several randomised controlled trials assessing the efficacy of the PAF antagonist lexipafant.

Kingsnorth and colleagues [163] demonstrated in a multicentre randomised controlled trial involving 83 patients with acute pancreatitis, that lexipafant therapy was able to ameliorate organ dysfunction and modulate serum IL8 concentrations. Similarly, a significant improvement in organ dysfunction scores was observed following lexipafant therapy in a randomised controlled trial of 50 patients with prognostically severe acute pancreatitis presenting within 72 hours of disease onset [164]. The final study demonstrating potential benefit was a phase III multicentre randomised controlled trial involving 290 patients with prognostically severe acute pancreatitis who were randomised to receive either lexipafant for 7 days or placebo [165]. When analysed on an intention to treat basis there was no difference in mortality (24/139 vs 18/151, p=.196), however subgroup analysis suggested a significant reduction in mortality in those patients treated within 48 hours of disease onset (20/98 vs 11/107, p=.04). Again lexipafant therapy led to significant improvements in organ dysfunction scores and reductions in serum IL8 concentrations.

On the basis of these clinical trials a large multinational trial has been undertaken in which patients were randomised to receive either high dose lexipafant, low dose lexipafant or placebo. Although recruitment to the trial has now been completed, formal publication of results is awaited. However, early reports suggest that lexipafant therapy does not improve outcome in patients with prognostically severe acute pancreatitis.

Anti-Inflammatory Cytokines

A number of cytokines have significant anti-inflammatory effects, and evidence is accumulating to suggest that they have important roles in the pathogenesis of acute pancreatitis.

Interleukin 10 (IL10) is an anti-inflammatory cytokine. Following induction of experimental acute pancreatitis increased synthesis of IL10 mRNA can be observed in the pancreas, liver and lungs, paralleling increases in TNFα mRNA expression [80]. As may be predicted, evidence derived from experimental models appears to suggests that IL10 has a protective role in acute pancreatitis. In animal models of acute pancreatitis the administration of exogenous IL10 reduces the cytokine response [166, 167] and the systemic effects of acute pancreatitis [168, 169]. Similarly, transfection with a plasmid-human IL10 construct protected against the subsequent initiation of acute pancreatitis in an animal model [170]. Conversely interventions to antagonise the actions of IL10 increase the severity of experimental acute pancreatitis. The administration of anti-IL10 antibody increases the production of TNFα and increases disease severity [166] whilst the elimination of IL10 through the use of an IL10 gene knockout mouse also increases disease severity [171].

However, although evidence from animal experimental models appears to confirm that IL10 has a protective role in acute pancreatitis, evidence from human acute pancreatitis suggests that the situation is more complex. Pezzilli and colleagues [172] initially reported that serum concentrations of interleukin 10 were lower in patients with severe acute pancreatitis when compared to those with mild disease, whilst Brivet and colleagues [89] observed that serum concentrations of IL10 were lower in non-survivors than survivors of an episode of severe acute pancreatitis. However, other studies have observed increased levels of serum IL10 in patients with severe disease [145, 173], whilst a positive correlation between serum IL10 concentrations and degree of organ dysfunction has also been observed [151].

The explanation for these contrasting observations is not clear. Although initial theories proposed that disease severity in critical illness states was due solely to excessive pro-inflammatory mediators it is probable that an excessive anti-inflammatory response is also detrimental. That is, outcome in critical illness states may be the consequence of a complex interaction between pro-inflammatory and anti-inflammatory mediators. Bone [76] has postulated the existence of a compensatory anti-inflammatory response syndrome (CARS) which is a consequence of the systemic inflammatory response and may help to perpetuate the critical illness state by inducing a state of immunosuppression encouraging opportunistic infection. In support of a complex interaction between pro-inflammatory and anti-inflammatory cytokines is the observation of Simovic and colleagues [174] that a decreased ratio of IL10 to IL6 or IL8 is important in determining severe disease.

Further research is required to elucidate the importance of both pro-inflammatory and anti-inflammatory mediators and their interaction in the pathogenesis of acute pancreatitis.

Leukocytes

An important part of the current model of the pathogenesis of severe acute pancreatitis is increased leukocyte activity. Both experimental and human observational studies appear to support the concept of increased leukocyte activity in severe acute pancreatitis.

A central role for neutrophils in the pathogenic mechanisms has been suggested by neutrophil depletion experiments in animal models. Depletion of granulocytes through the administration of hydroxyurea ameliorates the systemic effects of acute pancreatitis [175]. Likewise specific depletion of neutrophils through the administration anti-neutrophil antibody reduces the local [176-178] and systemic [177-179] effects in experimental models of acute pancreatitis. It should however be noted that

neutrophil depletion did not affect local pancreatic inflammation in a canine model of acute pancreatitis [180].

Studies in human patients would appear to confirm increased neutrophil activity in acute pancreatitis. Leukocyte scintigraphy has demonstrated increased uptake in the pancreas in patients with severe disease confirming localisation of activated leukocytes within the pancreas [181]. Similarly, several studies have confirmed increased activation of circulating neutrophils in patients with severe acute pancreatitis when compared to those with mild disease as evidenced by increased cell adhesion and superoxide production [142], increased neutrophil fluorescence with acridine orange [182] and luminol-enhanced chemoluminescence [183]. However, although increased neutrophil phagocytic activity has been reported [142], impaired phagocytosis has also been reported [182]. Further evidence of increased leukocyte activation in severe acute pancreatitis comes from the observation that patients with severe disease have significantly greater levels of serum polymorphonuclear elastase than those with mild disease [184].

Monocyte function in acute pancreatitis has also been studied. Isolated monocytes from patients with severe acute pancreatitis demonstrate increased secretion of TNFα, IL6 and IL8 [99], although cytokine secretion by peripheral blood mononuclear cells (monocytes and lymphocytes combined) is not increased [100]. In contrast however, other studies have suggested reduced monocyte function in severe acute pancreatitis. Larvin and colleagues [185] demonstrated that monocyte phagocytic function was reduced in patients with severe acute pancreatitis. Similarly Richter and colleagues [186] demonstrated down-regulation of monocyte human leukocyte antigen DR (HLA-DR) expression in those with severe disease, the suppression being greatest in those that died. The observations of decreased phagocytic function and HLA-DR expression would appear to further support the growing body of evidence that a compensatory anti-inflammatory response is important in determining outcome in acute pancreatitis.

Finally although a few studies have examined lymphocyte counts in acute pancreatitis, little is known regarding changes in function. In patients with severe acute pancreatitis there is a reduction in both the total lymphocyte count and the CD4+ lymphocyte count [183, 187, 188], although the CD4+:CD8+ ratio is maintained [183].

Endothelial Cells

Because of their relative inaccessibility the study of endothelial cell function in acute pancreatitis is difficult. Although direct evidence of endothelial cell activation in acute pancreatitis is available from studies in animal models, what little evidence that exists from human acute pancreatitis is indirectly determined through the measurement of serum soluble endothelial cell-derived molecules.

Intercellular Adhesion Molecule-1

Intercellular adhesion molecule-1 (ICAM-1) is a transmembrane glycoprotein belonging to the immunoglobulin gene superfamily [189]. In the normal (non-activated) state, endothelial cells demonstrate low levels of ICAM-1 cell surface expression, however following cellular activation expression is dramatically up-regulated. TNFα, IL1β, interferon γ and endotoxin are potent stimuli for endothelial ICAM-1 expression. Endothelial ICAM-1 cell surface expression is important in leukocyte-endothelial interactions during acute inflammation, enabling the endothelial cell to bind to leukocyte cell surface integrins [189].

Recent studies have suggested a role for ICAM-1 in the mediation of organ dysfunction in severe acute pancreatitis. Although an initial study did not observe an increase in pulmonary endothelial cell expression of ICAM-1 in an experimental model of acute pancreatitis [190], further studies have demonstrated that increased pulmonary ICAM-1 does occur [178, 191, 192], following increased pancreatic ICAM-1 expression [191]. The precise mechanisms through which ICAM-1 expression is

up-regulated in acute pancreatitis is not known, although both TNFα [193], and oxygen free radicals have been implicated [190]. Importantly, interventional studies aimed at blocking the action of ICAM-1 have suggested that up-regulation of ICAM-1 expression is a major event in the mediation of acute pancreatitis. The administration of a monoclonal antibody to ICAM-1 reduces both the local [191, 194, 195] and systemic effects of acute pancreatitis [191, 192, 195, 196]. Further, disease severity is less when acute pancreatitis is induced in mice that are deficient in ICAM-1 [178]. This improvement in outcome would appear to be the result of a perturbation of the leukocyte-endothelial cell interaction resulting in improved capillary blood flow, reduced leukocyte rolling, and stabilisation of capillary permeability [195].

Few data exist regarding the role of ICAM-1 in human acute pancreatitis although those which do are in concordance with observations derived from experimental models. Kaufmann and colleagues [197] have demonstrated that levels of serum ICAM-1 are significantly higher in patients with severe acute pancreatitis when compared to those with mild disease.

Selectin Family

The selectin family of cell adhesion molecules have an important early role in leukocyte margination and adhesion. The family consists of 3 related cell surface molecules; L-selectin, P-selectin and E-selectin, of which P-selectin and E-selectin are expressed on endothelial cells following an inflammatory stimulus [198].

A differential pattern of endothelial selectin expression appears to exist. P-selectin is stored pre-formed as a membrane component of the endothelial cell Weible-Palade body [199] and demonstrates rapid surface expression following cell stimulation by various agonists (e.g. thrombin, histamine, complement fragments [200]). In addition *de-novo* synthesis of P-selectin occurs following endothelial cell stimulation by various pro-inflammatory mediators including tumour necrosis factor-α, interleukin

1β and lipopolysaccharide [201]. In contrast E-selectin is not stored pre-formed in endothelial cells but is synthesised *de-novo* following an inflammatory stimulus (e.g. TNFα, IL1β and lipopolysaccharide [202, 203]). As a consequence of this requirement for synthesis, E-selectin cell surface expression lags behind P-selectin cell surface expression. Following cell surface expression, the interaction of P-selectin and E-selectin with their respective ligands on the cell surface of neutrophils and monocytes leads to leukocyte rolling and capture, the first steps in leukocyte migration [198]. Down-regulation of both P-selectin and E-selectin surface expression occurs mainly through internalisation [204, 205], however release of soluble forms of E-selectin and P-selectin also occurs [202, 206, 207]. These soluble forms of selectins can be measured in serum and may therefore be markers of endothelial cell activation [208, 209].

Evidence is accumulating from experimental models that suggests that the selectin family of adhesion molecules play important roles in acute pancreatitis. Up-regulation of P-selectin occurs within the lung following the induction of experimental acute pancreatitis [190, 210]. Interestingly, Lundberg and colleagues [210] observed a biphasic expression of P-selectin within the lung with the second peak coinciding with maximal E-selectin expression at 48 hours, and have suggested that the second P-selectin peak and the delayed E-selectin peak is mediated by pro-inflammatory cytokines. Finally the administration of an anti-P-selectin antibody ameliorates the acute lung injury in experimental acute pancreatitis [190].

In summary, data derived from both experimental animal models and from patients with acute pancreatitis would appear to support the hypothesis of a systemic inflammatory response characterised by the secretion of inflammatory mediators, the activation of leukocytes and their interaction with activated endothelial cells.

Tumour Necrosis Factor Gene Locus

The genes for both TNFα and TNFβ are located on the short arm of chromosome 6 and are closely associated with the major histocompatibility complex. Within the TNF gene locus a number of polymorphisms have been described [211] (Figure 1). Three restriction length polymorphisms (RFLP) have been described within the TNFβ gene (*NcoI, Asp*HI and *Eco*RI). Further, two single base polymorphisms in the promoter region of TNFα gene at positions -238 and –308 have been described. Finally, 5 microsatellites consisting of a variable copy number of dinucleotide repeats have also been described across the TNF gene locus.

TNF-308

A guanosine (G) to adenosine (A) base substitution may occur at position –308 of the TNFα promoter region giving rise to two possible alleles [212] (Figure 1 polymorphic site 4). The uncommon A base substitution has been designated as allele 2. Because of the position of the polymorphism within the promoter region of the TNFα gene it has been suggested that this polymorphism may have functional significance. Wilson and colleagues [213] have demonstrated that position -308 is the site of interaction for a DNA binding protein. Further, through the use of a plasmid mediated transient transfection system they demonstrated that allele 2 was associated with increased TNFα secretion, but was not associated with altered affinity for the DNA binding protein [213]. Likewise, Kroeger and colleagues [214] using a similar transfection system confirmed that the A base substitution was associated with an increase in TNFα production, however they observed differential affinity for DNA binding proteins within the 38 base pair region encompassing position -308. Further, Louis and colleagues [215] demonstrated that the uncommon A base substitution is associated with increased TNFα secretion in a whole blood culture system following

lipopolysaccharide stimulation. However, Brinkman and colleagues [216] did not observe differential secretion of TNFα between the two alleles within a transient transfection system. More recent data has however suggested that differential TNFα secretion associated with the TNF-308 polymorphism is both cell and stimulus specific [217].

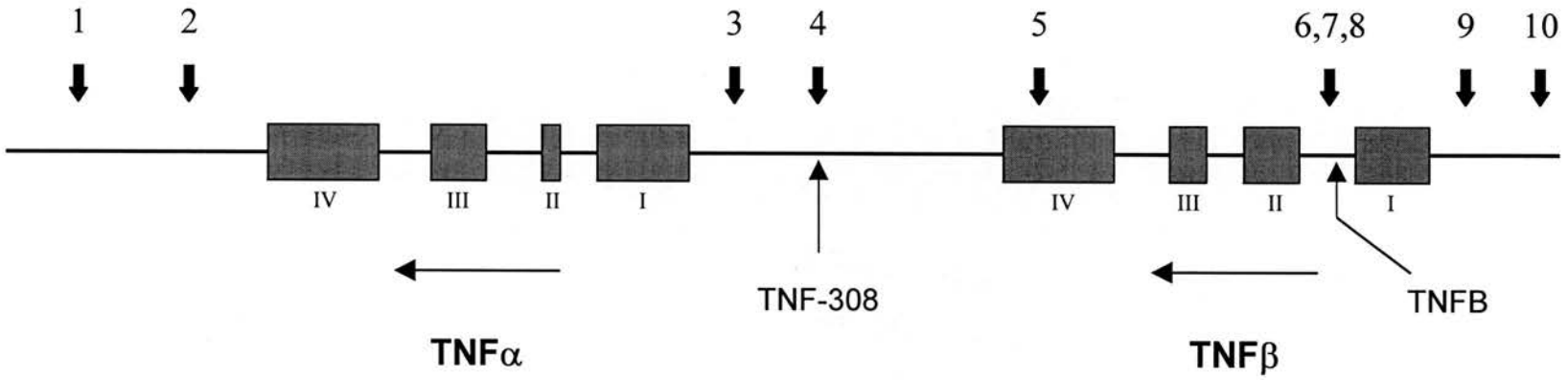
Although laboratory studies demonstrate an association between TNF-308 genotype and TNFα secretory phenotype, it is more significant that studies have demonstrated an association between TNF-308 genotype and outcome in cytokine-mediated acute inflammatory conditions. In children with meningococcal disease TNF-308 allele 2 is associated with increased disease severity and an increased risk of mortality [218]. Similarly TNF-308 allele 2 is associated with an increased risk of death or severe neurological sequelae in children suffering from malaria [219]. Further, in a multicentre observational study involving critically ill patients, carriage of TNF-308 allele 2 was associated with increased susceptibility to septic shock [220]; moreover, when controlled for other potential risk factors, carriage of TNF-308 allele 2 was associated with a 3.7 fold increased risk of death (95% C.I. 1.37 to 10.24) [220].

TNF-238

A guanosine (G) to adenosine (A) base substitution may occur at position –238 of the TNFα promoter region giving rise to two possible alleles [221] (Figure 1 polymorphic site 3). The uncommon A base substitution has been designated allele 2. The functional effects of this polymorphism are not clear. Recently Kaluza and colleagues [222] using a luciferase reporter gene assay demonstrated that allele 2 is associated with reduced production of TNFα. This is in contrast to previous *in vitro* studies which did not demonstrate an association between TNF-238 genotype and TNFα phenotype [223-225]. However, although the relationship between TNF-238 genotype and cytokine secretion is not clear, associations between the carriage of TNF-238 allele 2 and susceptibility to alcoholic steatohepatitis[226], chronic active hepatitis C [227] and hepatitis B infection [228] have been described. TNF-238 genotype is however not associated with disease severity in meningococcal disease [225].

TNFB

Within the first intron of the TNFβ gene an *NcoI* restriction fragment length polymorphism exists [229] (Figure 1 polymorphic site 7). This polymorphism has been correlated with an amino acid variation within the TNFβ sequence at position 26. Phytohemagglutinin stimulated peripheral blood mononuclear cells from individuals homozygous for allele 1 demonstrate increased secretion of TNFα, whilst phytohemagglutinin and endotoxin stimulated monocytes from individuals homozygous for allele 2 demonstrate increased secretion of IL1β and TNFβ [229-232]. However, despite poor association between TNFB genotype and cytokine secretory phenotype in isolated cell culture, significant associations between TNFB genotype and disease severity in critically ill patients have been described. In an observational study consisting of 40 patients with a diagnosis of sepsis, Stuber and colleagues [233] found that patients who were homozygous for TNFB allele 2 had increased levels of circulating TNFα. Further, those homozygous for allele 2 had significantly higher organ dysfunction scores and a significantly increased mortality rate. In a further study the same research group observed that following major trauma patients homozygous for TNFB allele 2 were at significantly higher risk of developing sepsis than patients with other TNFB genotypes [234].



Position	Nature of polymorphism	Reference
1	Dinucleotide repeat	235
2	Dinucleotide repeat	235
3	Single base variation	221
4	Single base variation	212
5	Single base variation	236
6	Dinucleotide repeat	237
7	Single base variation	230
8	Single base variation	238
9	Dinucleotide repeat	237
10	Dinucleotide repeat	237

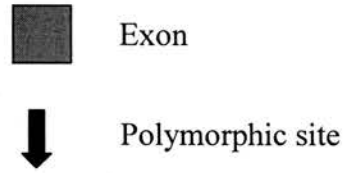


Figure 1: Tumour necrosis factor gene locus.

Interleukin 1 Family Gene Locus

The genes for all three members of the interleukin 1 family are located on the long arm of chromosome 2 [239] and are thought to arise from a common ancestral gene [121]. A number of polymorphisms have been described within the gene locus (Figure 2). Given that research has focussed mainly on the role of IL1β and IL1RA in the development of the systemic inflammatory response, consideration will be given to the polymorphisms within the IL1β and IL1RA gene loci which may give rise to phenotypic differences.

Interleukin 1β

Two polymorphisms have been described within the interleukin 1β gene (Figure 2).

Interleukin 1β -511

The first polymorphism consists of a cytosine (C) to thymine (T) base substitution at position –511 in the IL1β gene promoter region giving rise to two potential alleles [240] (Figure 2 polymorphic site 7). To date no studies have directly examined the effects of this polymorphism upon IL1β gene transcription. However, there does not appear to be a direct correlation between IL1β -511 genotype and serum IL1β in healthy individuals, although Hulkkonen and colleagues have suggested that an interaction exists between IL1β -511 genotype and an IL1α polymorphism which correlates with increased serum IL1β concentrations [241]. A further association has been described between IL1β -511 allele 2 and allele 2 of the IL1RA gene variable number tandem repeat polymorphism [242].

Interleukin 1β Taq1

The second polymorphism consists of a *TaqI* restriction fragment length polymorphism and occurs in exon 5 of the IL1β gene [243] (Figure 2 polymorphic site 6). This arises as a consequence of a cytosine (C) to thymine (T) single base variation and gives rise to the potential for 2 alleles. The *TaqI* polymorphism occurs in allele 1 and is the more common allele. The functional effects of the polymorphism are not entirely clear, although Pociot and colleagues [243] demonstrated increased secretion of IL1β by stimulated monocytes in individuals carrying allele 2. Similarly Wilkinson and colleagues [244] have demonstrated increased monocyte IL1β mRNA expression in individuals with carriage of allele 2 following stimulation with mycobacterium tuberculosis. However, carriage of IL1β *TaqI* allele 2 occurs less frequently in carriers of allele 2 of the IL1RA gene variable number tandem repeat polymorphism [242]. Therefore potential secretory differences may not be directly attributable to the interleukin 1β *TaqI* polymorphism, rather they may be related to IL1RA gene polymorphism. Regardless the IL1β *TaqI* polymorphism has been associated with clinical outcome in inflammatory bowel disease [245, 246], multiple sclerosis [247] and alcohol-induced liver disease [248].

Interleukin 1 Receptor Antagonist

The gene locus for interleukin 1 receptor antagonist has been designated IL1RN. Two polymorphisms exist within the IL1RN gene locus (Figure 2).

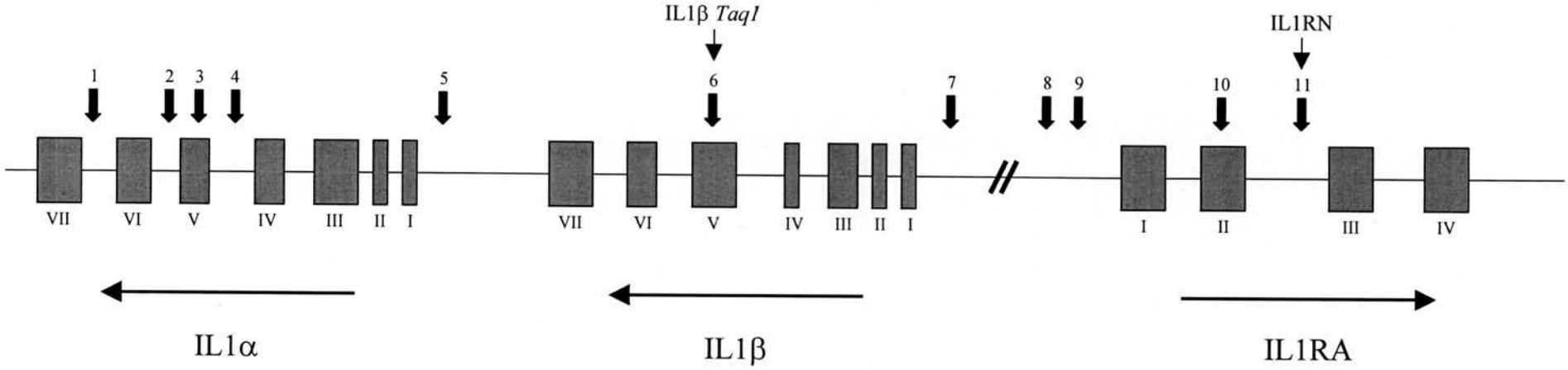
IL1RN (VNTR)

An 86 base pair variable number tandem repeat sequence exists in intron 2 of the IL1RN gene [249] (Figure 2 polymorphic site 11). Previous studies have identified the potential for 5 different alleles of which allele 2 is associated with increased IL1RA secretion [242, 250]. Although the mechanism through which this polymorphism

influences IL1RA secretion is not clear, a large number of studies have suggested that IL1RN (VNTR) allele 2 is associated with disease susceptibility and severity in a number of inflammatory conditions [251-255].

IL1RN (+2016)

A single base cytosine (C) to thymine (T) base substitution may occur at position +2016 within exon 2 giving rise to two potential alleles [256] (Figure 2 polymorphic site 10). These two alleles are in 100% linkage disequilibrium with the two most frequent alleles of IL1RN (VNTR) – allele 1 and allele 2. No studies have examined the potential for association between this polymorphism and disease states.



Position	Nature of polymorphism	Reference
1	Variable number tandem repeat	257
2	Dinucleotide repeat	258
3	Single base variation	259
4	Trinucleotide repeat	260
5	Single base variation	261
6	Single base variation	243
7	Single base variation	240
8	Tetranucleotide repeat	262
9	Dinucleotide repeat	263
10	Single base variation	256
11	Variable number tandem repeat	249

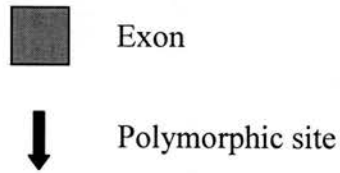


Figure 2: Interleukin 1 gene locus.

Critical Illness And The Intestinal Tract

The intestinal tract has many functions which are often interrelated and which may appear contradictory. For example, although the major role of the intestinal tract is the digestion and absorption of nutrients, it must also be both a physical and immunological barrier to molecules and organisms which if allowed to cross from the intestinal lumen could provoke a disease process. It is the failure of this intestinal barrier function which has been hypothesised to promote and sustain the systemic inflammatory response syndrome [264].

It has been hypothesised that a reduction in intestinal motility, altered intestinal microflora and failure of intestinal barrier function allow bacteria and, or, bacterial products to transfer from the intestinal lumen to the circulation [264]. This process has been termed bacterial translocation. Although the potential exists for a large number of bacteria or bacterial products to translocate, research has focussed on the role of endotoxin in the pathogenesis of the systemic inflammatory response. Endotoxin is a lipopolysaccharide derived from the outer membrane of gram-negative bacteria and has been demonstrated to be a potent pro-inflammatory stimulus. Other potential stimuli do exist; both peptidoglycan, a polysaccharide derived from gram-positive bacteria, and bacterial DNA can provoke a systemic inflammatory response [265].

Intestinal Permeability

Although intestinal function in critical illness can be relatively easily studied in animal models, it is necessarily more problematic in human patients, therefore indirect techniques for the assessment of intestinal function have been derived. One such method is the determination of intestinal permeability which may in turn be used as a surrogate marker of intestinal barrier function [266, 267]. Intestinal permeability relates to the passage of a solute across the intestinal epithelium through non-mediated diffusion. This is in contrast to absorption which is carrier-mediated. In any given state, permeability is dependent on the structure of the membrane, the

physicochemical properties of the solute and its interaction with the solvent. This therefore means that changes in intestinal barrier function occurring during critical illness states can be quantified by determining intestinal permeability through the measurement of the uptake of standardised non-metabolised probes. In practice, intestinal permeability is assessed by measuring the urinary excretion of orally administered test probes.

For the determination of intestinal permeability the perfect theoretical probe should be water-soluble, have first-order kinetics of permeation, be non-toxic, non-degradable and not metabolised either before, during or after permeating the intestinal tract. In addition because the tests rely on the quantification of the probe in urine following excretion, the probe should not be naturally present in urine, urinary excretion should be the sole mechanism of loss and urinary excretion should be complete following intravenous administration. Moreover measurement of the probe should be easy, sensitive and accurate.

Intestinal permeability is therefore quantified through a timed collection of urine following the ingestion of a fixed quantity of probe. Because other factors such as gastric emptying, intestinal blood flow and renal perfusion have the potential to confound the kinetics of the urinary excretion of the ingested probe, two probes are administered and it is the differential urinary excretion of these probes that is calculated in order to determine intestinal permeability [266, 267]. Although a number of probes have been used it is conventional to use a monosaccharide and a disaccharide together.

Critical Illness States And Changes In Intestinal Permeability

A number of studies have confirmed that, as postulated, intestinal barrier function is altered during the systemic inflammatory response.

O'Dwyer and colleagues [268] demonstrated that increased intestinal permeability occurs in a human experimental model of the systemic inflammatory response syndrome. Following the systemic administration of endotoxin to 12 healthy volunteers an increase in intestinal permeability to lactulose and mannitol was observed, with the degree of intestinal dysfunction correlating with the degree of systemic perturbation as manifested by serum norepinephrine concentration and white blood cell count.

Similar changes in intestinal permeability have been observed in critically ill patients. Johnston and colleagues [269] observed increased intestinal permeability to lactulose and rhamnose in 20 patients with sepsis when compared to healthy individuals. Likewise, Harris and colleagues [270] demonstrated increased intestinal permeability to lactulose and mannitol in 16 critically ill patients, although there did not appear to be any correlation between intestinal permeability and disease severity. Finally Ziegler and colleagues [271] reported that patients with infected burns demonstrated increased intestinal permeability to lactulose and mannitol correlating with the severity of the burn infection. Interestingly patients with simple non-infected burns did not have increased intestinal permeability.

It would therefore appear that the development of a systemic inflammatory response syndrome is associated with increased intestinal permeability. However, it should be noted that studies assessing intestinal permeability in critical illness do not provide direct evidence regarding the role of the intestinal tract in sustaining the systemic inflammatory response, that is, it is not clear whether the changes observed in the intestinal tract are solely a manifestation of the systemic inflammatory response or whether the changes are important in mediating the disease process itself.

Effects Of Enteral Nutrition On Intestinal Permeability

The mechanisms through which intestinal dysfunction arises in critical illness states are not clear, although a number of factors have been postulated. These factors

include splanchnic ischaemia/reperfusion, sepsis, therapeutic intervention and loss of enteral nutrition [272]. Unfortunately not all of these factors can be easily modified by the treating clinician, however because of the potential for relatively simple intervention, it is the loss of enteral nutrition which deserves special attention.

In an observational study involving healthy volunteers and malnourished patients, Maxton and colleagues [273] observed that starvation for a period less than 36 hours did not increase intestinal permeability but did reduce intestinal absorption, whilst longer periods of starvation increased intestinal permeability and reduced intestinal absorption. However, the alteration in intestinal permeability does not appear to be due solely to the loss of adequate nutrition. Buchman and colleagues [274] observed that healthy individuals maintained on parenteral nutrition instead of normal enteral nutrition also demonstrated increased intestinal permeability. These observations together suggest that in order to maintain normal intestinal function adequate nutrition needs to be provided by the enteral route. It would therefore seem logical that as part of any therapeutic management strategy designed to minimise intestinal dysfunction in critical illness states, adequate nutrition should be delivered through the enteral route.

However, only a few randomised clinical trials have examined the effect of enteral nutrition upon intestinal dysfunction in critical illness states. Hadfield and colleagues [275] assessed the benefit of enteral nutrition in maintaining normal intestinal function in patients with a critical illness. In total 24 patients were randomised to receive either enteral or parenteral nutrition. At the start of the trial intestinal permeability was increased in both groups. However, by day 9 a significant reduction in intestinal permeability was observed in the group receiving enteral nutrition, whilst no change in intestinal permeability was observed in those receiving parenteral nutrition.

Further, in a randomised trial involving 30 patients undergoing elective laparotomy and intestinal resection, when compared to standard care with intravenous crystalloid, the introduction of enteral nutrition in the immediate post operative

period was associated with maintained intestinal permeability [276]. However, in a similar randomised trial involving patients undergoing elective resection of upper gastro-intestinal malignancy, Brooks and colleagues [277] found that intestinal permeability was increased on the first post-operative day in all patients and returned to normal on the fifth post-operative day irrespective of whether they did or did not receive enteral nutrition.

In summary, published studies examining the effect of the administration of enteral nutrition upon intestinal permeability in critical illness states are inconclusive. However, regardless of the effects of enteral nutrition on markers of intestinal function such as intestinal permeability, changes in disease mortality and morbidity are the most important outcome measures. Therefore evidence of improved clinical outcomes must come from well constructed randomised trials before it can be conclusively stated that the delivery of nutrition via the enteral route is required in order to provide the optimum outcome in patients with a critical illness.

Trials Of Enteral Nutrition In Critical Illness

To date a large number of trials have been undertaken in an attempt to assess the potential benefit of enteral nutrition in patients with trauma, burns, critical illness states or following elective gastro-intestinal surgery (Table 7 and Table 8). Although the majority appear to suggest that enteral nutrition provides significant benefit, methodological differences and flaws may be identified within and between published trials with the result that definite conclusions may not be drawn.

Firstly the majority of published trials contain relatively few study subjects thereby rendering the trials statistically under-powered. Secondly in some trials the nutritional regimes administered to each randomised group were not isocaloric [278] or isonitrogenous [278-282]. Thirdly the methods of nutritional support were not consistent between trials, with comparison between enteral nutrition and parenteral nutrition as well as enteral nutrition and the fasting state [278, 279, 283-288]. Finally the enteral

nutrition formulation under investigation is not consistent between trials, with more recent studies employing supplemented nutritional formulae which have been promoted to have immune-enhancing properties with proposed further improvement in clinical outcome [278-282, 285-287].

In an attempt to circumvent these difficulties, several groups have carried out meta-analysis of published enteral nutrition trials.

The first meta-analysis was carried out by Moore and colleagues [289] and included data derived from two published randomised trials and six previously unreported randomised trials all of which were designed to assess the benefit of enteral nutrition when compared to parenteral nutrition in surgical patients. In total, outcome data for 230 patients who had undergone both elective and emergency surgery were available. When analysed on an intention-to-treat basis, patients receiving enteral nutrition had significantly fewer infectious complications (16% enteral vs 29% parenteral p=.03), with the greatest benefit occurring in patients with major trauma.

Likewise Heys and colleagues [290] carried out a meta-analysis of 11 published randomised trials assessing the beneficial effects of immune-enhancing enteral nutrition when compared to standard enteral nutrition. The subsequent analysis of data derived from 1009 patients demonstrated that the use of an immune-enhancing diet was associated with a significant reduction in major infectious complications, that is pneumonia, major wound infection, intra-abdominal collection and septicaemia (odds ratio 0.47, 95% CI 0.32 to 0.70). However, although there was no significant difference between the two patient groups with respect to mortality, there was a trend to increasing mortality in those patients receiving supplemented enteral nutrition (OR 1.77 95% CI 1.00 to 3.12).

Similarly Beale and colleagues [291] carried out a meta-analysis of twelve published studies assessing the benefits of supplemented enteral nutrition formulae. Eight of these twelve studies were included in the meta-analysis carried out by Heys and colleagues [290]. Beale analysed outcome data derived from 1,482 patients who had

been randomised to either standard enteral nutrition or a supplemented (immune-enhancing) nutritional formula. On an intention-to-treat basis, immune-enhancing nutritional formulae were associated with a significant reduction in septic complications (OR 0.60 95% CI 0.28 to 0.83) and a significant reduction in the number of days of ventilation (2.6 days reduction 95% CI 0.1 to 5.1). However, immune-enhancing nutrition was not associated with an improvement in the mortality rate (OR 1.05 95% CI 0.78 to 1.41).

Overall, results from published trials suggest that enteral nutrition is associated with a significant reduction in infectious complications, although as yet there is no definite evidence demonstrating that this results in a reduction in mortality. Supplemented or immune-enhancing nutrition may provide further outcome benefits.

Author	Year	Type of patient	Intervention	Number of patients	Outcomes
Moore[288]	1986	Trauma	Enteral nutrition vs no nutrition	63	Significant improvement in nitrogen balance and reduction in septic complications in enteral nutrition group.
Moore[283]	1989	Trauma	Enteral vs parenteral nutrition	59	Significant reduction in infectious complications in enteral nutrition group
Kudsk[284]	1992	Trauma	Enteral vs parenteral nutrition	98	Significant reduction in infectious complications. Benefits greatest in most severely injured patients
Eyer[292]	1993	Trauma	Early enteral vs late enteral	52	No significant difference in metabolic response or clinical outcome

Table 7: Randomised trials containing at least 50 subjects designed to assess the benefits of standard enteral nutrition.

Author	Year	Type of patient	Routes of feed delivery	Contents of supplemented feed	Number of patients	Outcome
Daly[280]	1992	Elective surgery	Enteral vs enteral	Arginine, ω-3 fatty acids, nucleotides	85	Reduction in infectious and wound complications in the supplemented group.
Daly[287]	1995	Elective surgery	Enteral vs enteral	Arginine, ω-3 fatty acids, nucleotides	60	Reduction in major post-operative complications in the supplemented group.
Moore[281]	1994	Trauma	Enteral vs enteral	Arginine, glutamine, ω-3 fatty acids, nucleotides	98	Reduced rate of intra-abdominal collections and MODS in the supplemented group.
Bower[279]	1995	Trauma, surgery or sepsis	Enteral vs enteral	Arginine, ω-3 fatty acids, nucleotides	296	No significant difference on intention to treat.
Senkal[286]	1997	Elective abdominal surgery	Enteral vs enteral	Arginine, ω-3 fatty acids, nucleotides	154	Significant reduction in "late" complications (after 5th post-operative day) but not in overall complication rate in the supplemented group.
Saffle[282]	1997	Burns	Enteral vs enteral	Arginine, glutamine, ω-3 fatty acids, nucleotides	50	No significant difference in outcome.
Atkinson[293]	1998	Critical illness	Enteral vs enteral	Arginine, glutamine, ω-3 fatty acids, nucleotides	390	No significant difference on intention to treat. Potential benefit if successful feeding achieved in the supplemented group.
Houdijk[285]	1998	Trauma, surgical	Enteral vs enteral	Glutamine	72	Significant reduction in chest infection, bacteraemia and sepsis syndrome in the supplemented group.
Heslin[278]	1997	Elective upper gastro-intestinal surgery	Enteral vs no nutritional support	Arginine, glutamine, ω-3 fatty acids, nucleotides	195	No significant difference in outcome.
Braga[294]	1998	Elective upper gastro-intestinal surgery	Supplemented enteral vs standard enteral vs parenteral	Arginine, ω-3 fatty acids, nucleotides	166	Significant reduction in severity of infection between the supplemented enteral and the parenteral nutrition groups.

Table 8: Randomised trials containing at least 50 subjects designed to assess the benefits of supplemented (immune-enhancing) enteral nutrition formulations.

As with other organ systems, the intestinal tract is affected early in the course of acute pancreatitis. However, it would appear that the intestinal tract is not merely a bystander in the pathogenesis of severe disease, rather evidence is accruing to implicate intestinal tract dysfunction as a major factor in maintaining the systemic inflammatory response which characterises severe acute pancreatitis.

Alterations In Intestinal Tract Function In Experimental Acute Pancreatitis

The majority of data relating to the intestinal tract in acute pancreatitis have been obtained from animal models, although data do exist relating to human acute pancreatitis and this is in concordance with the animal model-derived evidence.

During the early stages of experimental acute pancreatitis, intestinal blood flow is reduced [295-297], to the extent that in severe disease splanchnic hypo-perfusion results in both mucosal and subserosal ischaemia [298]. It is likely that this period of ischaemia along with the subsequent reperfusion injury is a major factor in initiating intestinal tract dysfunction [272].

Following the initial insult, the interval between migrating myoelectric complexes increases [299] resulting in decreased intestinal motility and increased intestinal transit time [300, 301]. This decrease in intestinal motility allows for an alteration of the intestinal microflora and colonisation of the normally sterile upper intestinal tract with bacteria [300, 302]. In addition, intestinal epithelial cell dysfunction occurs resulting in failure of intestinal barrier function. This failure of intestinal barrier function is in part manifested by an increased permeability to non-absorbable molecules [297], the increase in which is correlated with the severity of the acute pancreatitis [303]. Accompanying the increase in intestinal permeability to

non-absorbable molecules there is a reduction in the normal active transport of other molecules [297].

Effects Of Intestinal Tract Dysfunction In Experimental Acute Pancreatitis

It has been hypothesised that a reduction in intestinal motility, altered intestinal microflora and failure of intestinal barrier function allow bacteria and/or, bacterial products to transfer from the intestinal lumen to the circulation [264]. This process has been termed bacterial translocation, and has been proposed to be important in maintaining a systemic inflammatory response. However, in acute pancreatitis translocation of bacteria from the intestinal tract may have consequences other than the maintenance of a systemic inflammatory response. Evidence has accumulated that the intestinal tract is the origin of organisms involved in the development of infected pancreatic necrosis complicating acute pancreatitis [304].

The process of bacterial translocation is relatively easily studied in animal models of acute pancreatitis, with culture of tissue including mesenteric lymph nodes allowing quantification of bacterial translocation. Many studies have confirmed the process of bacterial translocation in experimental acute pancreatitis [300-302, 305, 306], and its association with decreased intestinal motility [300, 301] and altered intestinal microflora [300, 302].

It is most probable that bacterial translocation to mesenteric lymph nodes occurs through the intestinal lymphatic system, but the routes through which bacteria spread from the intestinal tract to the pancreas are less clear [307]. Medich and colleagues [308] demonstrated spread of fluorescent beads from the gut to the pancreas through the transperitoneal route. In contrast, Arendt [309] suggested that the peritoneal cavity acted as a barrier to transperitoneal spread. However, in a series of experiments Widdison and colleagues [310] have demonstrated that colonisation of pancreatic

necrosis can occur through haematogenous spread, transperitoneal spread or retrograde spread along the pancreatic duct.

Further evidence supporting the importance of the intestinal tract in the pathogenesis of infected pancreatic necrosis comes from experiments assessing the effects of intestinal decontamination. Foitzik and colleagues [311] demonstrated that oral antibiotics altered normal intestinal flora and when combined with intravenous antibiotics led to a reduction in rates of pancreatic infection. Similarly in a mouse model of severe acute pancreatitis, therapy with oral bacitracin, metronidazole and neomycin resulted in reduced rates of positive bacterial cultures from samples of peripheral blood, ascites, spleen and pancreas. Further, Gianotti and colleagues [312] evaluated several different gut decontamination regimes in a mouse model of acute pancreatitis. Although all decontamination regimes resulted in significant reductions in intestinal bacterial flora, not all regimes were associated with decreased rates of bacterial translocation and reduced mortality rates, suggesting that the benefits of gut decontamination do not arise solely from the elimination of pathogenic intestinal bacteria. Finally, simple colonic lavage has been demonstrated to reduce bacterial translocation in a rat model of acute pancreatitis [313].

The Intestinal Tract In Human Acute Pancreatitis

In human acute pancreatitis, direct assessment of splanchnic blood flow is difficult. Using a regional catheter and dilution dye technique, Ruokenen and colleagues [314] demonstrated that following fluid resuscitation splanchnic blood flow in patients with severe acute pancreatitis is similar to that in patients prior to elective major abdominal surgery. However, splanchnic oxygen consumption was significantly increased in patients with severe acute pancreatitis suggesting that despite maintained splanchnic blood flow, oxygen delivery may not be sufficient.

Further data relating to intestinal perfusion in acute pancreatitis is derived from the use of intestinal pH manometry. During episodes of intestinal hypoperfusion the

intra-mucosal pH falls and this may be measured providing indirect evidence of intestinal ischaemia. Juvonen and colleagues [315] demonstrated a fall in gastric intra-mucosal pH within the first 48 hours of presentation in all patients with acute pancreatitis, but did not observe significant differences in intra-mucosal pH between patients with mild or severe disease. In contrast Soong and colleagues [96] observed significantly lower gastric intra-mucosal pH measurement in patients with severe acute pancreatitis when compared to those with mild disease. Similarly within the group of patients with severe acute pancreatitis, prolonged falls in intra-mucosal pH correlates with poor outcome [151, 316].

In addition to alterations in intestinal perfusion, patients with severe acute pancreatitis demonstrate increased intestinal permeability. Ammori and colleagues [317] quantified intestinal permeability using polyethyleneglycol (PEG) 400 and PEG 3350. Using this technique Ammori demonstrated increased intestinal permeability in patients with severe acute pancreatitis. Moreover the increase in intestinal permeability correlated with the presence of organ dysfunction. Further, an association existed between increased intestinal permeability and endotoxaemia, an observation in keeping with the concept of bacterial translocation.

Other studies have also examined the presence of endotoxaemia in acute pancreatitis. Foulis and colleagues [318] were the first to observe that endotoxaemia occurs more frequently in patients with severe acute pancreatitis than those with mild disease, their results subsequently being confirmed [92, 96]. However, endotoxaemia is not observed to occur in all cases of severe acute pancreatitis [92, 96, 151, 318] and therefore it has been suggested that endotoxaemia may be transient and consequently missed by intermittent sampling. As a result serum anti-endotoxin antibody concentrations have been proposed as an indirect measure of endotoxin exposure. Windsor and colleagues [319] observed that a significant fall in the serum anti-endotoxin core antibody class IgG concentration was associated with severe disease, whilst Soong and colleagues [96] demonstrated that a fall in anti-endotoxin core antibody class IgM concentration was associated with poor outcome. Falls in serum anti-endotoxin core antibody concentrations would be in keeping with consumption of endogenous

antibodies following exposure to endotoxin exposure and would again support the concept of bacterial translocation.

Further evidence demonstrating the importance of the intestinal tract in the pathogenesis of severe acute pancreatitis is provided by a randomised controlled trial assessing the benefit of selective gut decontamination in patients with severe acute pancreatitis. In a multicentre trial, Luiten and colleagues [320] randomised 102 patients with prognostically severe acute pancreatitis as defined by multiple laboratory criteria (Glasgow [321]) and/or contrast enhanced-CT scoring (Balthazar [322]) to either standard conventional therapy or standard conventional therapy supplemented with selective gut decontamination using oral and rectal colistin, amphotericin and norfloxacin. In addition, patients in the selective gut decontamination group received intravenous cefotaxime until aerobic gram negative bacteria were eliminated from the oral cavity and rectum as determined by serial bacterial culture Although there was a reduction in mortality from 35% in the control group to 22% in the selective decontamination group it did not reach significance (p=.19). However, when allowing for differences in disease severity, multivariate analysis suggested a significant survival benefit following gut decontamination (p=.048). Encouragingly selective gut decontamination reduced rates of pancreatic infection when compared to the control group (38% vs 18%, P=.03). Further, re-colonisation of the intestinal tract by gram negative bacteria following the implementation of the selective gut decontamination regime was a significant risk factor for the subsequent development of pancreatic infection by the same organism [323].

Enteral nutrition in acute pancreatitis

Part of the accepted management of acute pancreatitis is the institution of a nil-by-mouth regime. Prior to the newer models of acute pancreatitis it had been hypothesised that organ dysfunction in acute pancreatitis was due to the systemic action of activated digestive enzymes released into the circulation from the inflamed pancreas. Fasting was therefore believed to limit detrimental pancreatic secretion.

However, given the current hypotheses regarding the role of the systemic inflammatory response in mediating organ dysfunction in acute pancreatitis and the putative importance of intestinal tract dysfunction in maintaining this inflammatory response, the rationale for the nil-by-mouth regime has been challenged. It is significant therefore, that the introduction of enteral nutrition would appear to reverse the changes that have been postulated to be important in maintaining the systemic inflammatory response in acute pancreatitis. In an animal model of acute pancreatitis, Kotani and colleagues [324] demonstrated that when compared to parenteral nutrition the introduction of enteral nutrition maintains intestinal villus height and enterocyte cell proliferation, maintains lymphocyte function and decreases bacterial translocation as manifested by mesenteric lymph node cultures and systemic plasma endotoxin concentration. However, although these observations would appear to be consistent with recent hypotheses and suggest significant benefit, before it can be definitively concluded that the use of enteral nutrition in acute pancreatitis provides significant clinical outcome benefits, evidence from randomised clinical trials is required. Importantly therefore, three small randomised clinical trials have recently been published which provide an early assessment of the benefits of enteral nutrition in acute pancreatitis.

McClave and colleagues [325] randomised 30 patients with acute pancreatitis to receive either early enteral nutrition through an endoscopically placed nasojejunal feeding tube, or, parenteral nutrition following central or peripheral venous cannulation. Enteral nutrition appeared to be well tolerated with no patients developing a significant complication from the study intervention. Importantly enteral nutrition was able to supply a similar calorific intake to parenteral nutrition. However, patients had relatively mild disease and thus no significant differences in clinical outcome were observed, although enteral nutrition was associated with a significant reduction in the cost of patient care.

In a similar study Windsor and colleagues [326] randomised 34 patients with acute pancreatitis to receive either early enteral nutrition or parenteral nutrition. The route of delivery of the enteral nutrition was dependent on the prognostic severity of the

episode. In patients with prognostically mild disease enteral nutrition was taken orally, whilst enteral nutrition was delivered through a radiologically positioned nasojejunal tube in those with prognostically severe disease. The introduction of early enteral nutrition was associated with a significant reduction in serum C-reactive protein concentrations and APACHE II scores. Further, the use of parenteral nutrition was associated with a reduction in serum anti-oxidant potential, which is consistent with the presence of an on-going inflammatory response.

In contrast to the other two studies, all patients in the randomised controlled trial reported by Kalfarentzos [327] had prognostically severe disease. In total 38 patients were randomised to receive either enteral nutrition or parenteral nutrition. Again enteral nutrition was delivered distal to the ligament of Trietz through a radiologically screened feeding tube. Even in this population of patients with severe disease enteral nutrition was well tolerated, with the protein and caloric intake equalling that administered to the patients receiving parenteral nutrition. This ability to provide adequate nutrition via the enteral route appeared to translate into a clinical benefit. In those patients receiving enteral nutrition there were significantly fewer complications and a significant reduction in the number of infectious episodes. Further, the cost of nutritional support in the enteral feeding group was one third of that in the parenteral nutrition group.

Hypothesis

Current models of acute pancreatitis hold that remote organ dysfunction in acute pancreatitis is a consequence of the development of a systemic inflammatory response which is characterised by the interaction of activated leukocytes, activated endothelial cells, and the secretion of inflammatory mediators such as cytokines.

Although a body of evidence exists supporting the presence of activated leukocytes and increased pro-inflammatory cytokine secretion in severe acute pancreatitis, little evidence exists regarding the role of the endothelial cell. This is due in part to the inaccessibility of endothelial cells. However, the endothelial cell surface adhesion molecules, E-selectin and P-selectin, may be readily assayed in serum following endothelial cell surface expression thereby providing a measure of endothelial cell function. Therefore in order to provide information regarding the role of the endothelial cell in the pathogenesis of severe acute pancreatitis the kinetics of serum soluble E-selectin and P-selectin have been determined in patients with mild and severe acute pancreatitis. It is hypothesised that serum soluble E-selectin and P-selectin concentrations will be increased in patients with severe acute pancreatitis when compared to those with mild disease reflecting the increased endothelial cell activation that occurs in those patients with severe disease.

Although it is now accepted that organ dysfunction in patients with severe acute pancreatitis is mediated in part by increased pro-inflammatory cytokine secretion, the mechanisms which result in differential secretion of pro-inflammatory cytokines between individuals with mild or severe acute pancreatitis are not known. As in other biological systems, cytokine secretion is determined by both environmental and genetic factors. Although environmental factors may be important in mediating increased cytokine secretion in severe acute pancreatitis none have been proven to be the primary mechanism through which differential cytokine secretion occurs. However, polymorphisms have been identified within cytokine gene loci which have been demonstrated to have functional relevance to levels of cytokine secretion, and moreover, have been demonstrated to have clinical significance in some

inflammatory conditions. Such polymorphisms have been demonstrated in the tumour necrosis factor and interleukin 1 family gene loci. Therefore the relationship between polymorphisms within the tumour necrosis factor and interleukin 1 family gene loci, and disease severity in acute pancreatitis have been determined. It is hypothesised that gene polymorphisms which are associated with increased pro-inflammatory cytokine activity will occur more frequently in patients with severe acute pancreatitis. In addition, because the polymorphisms studied may not be the most important polymorphisms with regard to determining cytokine secretion, cytokine secretory phenotype has been determined following recovery from an episode of acute pancreatitis. It is hypothesised that following a standard inflammatory stimulus, increased leukocyte pro-inflammatory cytokine secretion would be observed in those patients with previous severe acute pancreatitis when compared to those with previous mild disease.

Finally, recent hypotheses have proposed that the maintenance of the systemic inflammatory response in critical illness states is a consequence of intestinal dysfunction which results in translocation of bacteria and bacterial products which are potent inflammatory stimuli. Intestinal dysfunction in critical illness states is believed to be due in part to the loss of nutrition following the implementation of a nil-by-mouth regime. Further, it has been proposed that the introduction of enteral nutrition is able to reverse intestinal dysfunction in critical illness states, thereby ameliorating the inflammatory response. Therefore within the confines of a randomised controlled trial, the effect of early enteral nutrition upon the inflammatory response in patients with severe acute pancreatitis has been examined. It is hypothesised that the introduction of early enteral nutrition will improve intestinal barrier function, thereby reducing bacterial translocation resulting in a reduction in the systemic inflammatory response.

In summary the hypotheses tested within this thesis are:

1. That serum soluble E-selectin and P-selectin concentrations will be increased in patients with severe acute pancreatitis when compared to those with mild disease as a consequence of increased endothelial cell activation.

2. That cytokine gene polymorphisms associated with increased cytokine secretion will have significant associations with disease severity in acute pancreatitis

3. That pre-determined differential leukocyte cytokine secretion exists between individuals who have mild or severe acute pancreatitis.

4. That the introduction of early enteral nutrition in patients with prognostically severe acute pancreatitis will improve intestinal barrier function, resulting in a reduction in bacterial translocation, thereby ameliorating the systemic inflammatory response.

Materials And Methods

Kinetics Of Serum Soluble E-Selectin And P-Selectin In Acute Pancreatitis

Patients

Eighteen patients admitted to the Royal Infirmary of Edinburgh with acute pancreatitis were studied. Consistent with the Atlanta consensus conference criteria [1], a diagnosis of acute pancreatitis was made on the basis of a history consistent with acute pancreatitis and a serum amylase concentration greater than 3 times the upper limit of normal, or radiological (computed tomography) evidence of acute pancreatitis. All patients within the present study had serum amylase concentrations greater than 3 times the upper limit of normal, moreover all patients with severe acute pancreatitis had evidence of acute pancreatitis on computed tomographic scanning. Data were collected prospectively on all patients allowing retrospective categorisation of disease severity according to criteria defined by the Atlanta consensus conference [1]. Because the present study aimed to determine levels of endothelial dysfunction attributable to acute pancreatitis rather than other disease processes, only patients with primary multiple organ dysfunction as defined by current criteria set by the American College of Chest Physicians/Society of Critical Care Medicine were included in the group of patients with severe disease [328]. The patient characteristics are given in Table 9. The individual characteristics of those patients with severe disease is given in Table 10. All patients with severe disease had evidence of primary organ dysfunction; 5 (56%) patients required ventilation and renal replacement therapy during the first three days of their admission. In total 6 patients (67%) with severe disease died of complications of acute pancreatitis during their admission. The median time from admission to death was 38 days (range 2-135 days). In contrast no patient with mild acute pancreatitis had evidence of organ dysfunction nor did any patient with mild disease succumb to their illness. As current evidence indicates that the cytokine-mediated systemic inflammatory

response syndrome responsible for organ dysfunction in acute pancreatitis occurs in the initial stages of the disease and as the major focus of potential therapeutic interventions is to modulate this early inflammatory response, the initial three days following admission were selected as the study period.

Disease severity [1]	Mild	Severe	
	n=9	n=9	
Age	39 (33-72)	59 (44-66)	Mann-Whitney U p=.566
Sex	4M:5F	6M: 3F	χ^2=0.90, p=.343
Aetiology			
Gallstone	5	3	
Alcohol	4	4	
Idiopathic	0	1	
Other	0	1	χ^2=2.50, p=.475
Delay from onset of pain to first sample (hours)	13 (10-21)	21 (12-31)	Mann-Whitney U p=.377
APACHE II score [329]	5 (3-7)	20 (16-26)	Mann-Whitney U p<.001
Glasgow score [321]	2 (0-2)	6 (5-6)	Mann-Whitney U p<.001

Table 9: Characteristics of patients in whom serum soluble selectin concentrations were determined.
Stated values are median (interquartile range).

Sex	Age	Aetiology	APACHE II Score [329]	Glasgow Score [321]	Organ Dysfunction	Organ Support	Outcome	Mode Of Death
Male	62	Alcohol	30	6	Respiratory, renal, cardiovascular, DIC	Ventilation, renal replacement therapy, inotropes	Non-survivor	MODS
Male	24	Alcohol	32	5	Respiratory, renal, cardiovascular, metabolic acidosis,	Ventilation, renal replacement therapy, inotropes	Non-survivor	Intra-peritoneal haemorrhage
Male	65	Gallstone	19	4	Respiratory		Survivor	
Female	59	Post-ERCP	13	6	Respiratory		Survivor	
Male	84	Gallstone	21	6	Respiratory, renal		Non-survivor	MODS
Male	31	Alcohol	20	5	Respiratory, renal	Ventilation, renal replacement therapy	Non-survivor	Intra-peritoneal haemorrhage
Female	69	Idiopathic	25	7	Respiratory, renal, cardiovascular, DIC, metabolic acidosis	Ventilation, renal replacement therapy, inotropes	Non-survivor	Intra-peritoneal haemorrhage
Female	49	Gallstone	8	5	Respiratory		Survivor	
Male	58	Alcohol	18	6	Respiratory, renal, cardiovascular, DIC, metabolic acidosis	Ventilation, renal replacement therapy, inotropes	Non-survivor	MODS

Table 10: Characteristics of patients with severe acute pancreatitis in whom serum soluble selectin concentrations were determined.

ERCP-endoscopic retrograde cholangiopancreaticogram. DIC-disseminated intravascular coagulation. MODS-multiple organ dysfunction syndrome.

Sample Collection And Assay

After obtaining informed consent a venous blood sample was collected into a blood tube without additive (Monovette® Sarstedt, Numbrecht Germany) and allowed to clot. Samples were then centrifuged at 900g for 10 minutes. Serum was then removed and stored at -70°C until analysis. Samples were collected on days 1, 2 and 3 of the in-patient stay. One patient in the severe group died on day 2. Because haemoconcentration may affect serum soluble selectin concentrations, and the platelet count may correlate with serum soluble P-selectin concentrations [330], venous blood was also collected into ethylenediaminetetraacetic acid (EDTA) coated tubes (Monovette® Sarstedt, Numbrecht Germany) for full blood count measurement.

Serum soluble E-selectin and P-selectin concentrations were measured by enzyme-linked immunosorbent assay (R&D Systems, Abingdon UK). The lower limit of detection for P-selectin and E-selectin measurement was 19ng/mL and 21ng/mL respectively. The P-selectin ELISA intra-assay and inter-assay coefficients of variance were 2.4% and 4.6% respectively. The E-selectin ELISA intra-assay and inter-assay coefficients of variance were 4.4% and 6.9% respectively.

Full blood count measurements were carried out using an automated cell counter (Sysmey NE8000, Toa Medical Electronics, Japan).

Statistical Analysis

Patient characteristic variables are presented as median and interquartile range. Serum soluble selectin and full blood count results are presented as mean and standard error of the mean. Categorical data were compared using the χ^2 test. Continuous data were compared using the Mann-Whitney U-test. Comparison of repeated measurements between the two patient groups was carried out using analysis of variance techniques (ANOVA). Significance was taken at the p=.05

level. All analyses were carried out using the Statview 5.0 software package (SAS Institute Inc, North Carolina).

Study Population

All patients admitted to the Royal Infirmary of Edinburgh with a diagnosis of acute pancreatitis were eligible for study. The diagnosis of acute pancreatitis was based on a history consistent with acute pancreatitis and serum amylase levels greater than three times the upper limit of normal, or, radiological (computed tomography) evidence of acute pancreatitis. Between September 1996 and May 1998 a total of 190 patients consented to enrolment in the study. Data on the clinical course were collected prospectively allowing categorisation of disease severity according to criteria defined by the Atlanta Consensus Conference [1]. Of the 190 patients enrolled, 113 had mild disease whilst the remaining 77 had severe disease. Further, in 169 patients who were admitted to the Royal Infirmary of Edinburgh at the onset of their illness it was possible to quantify disease severity using the modified Glasgow prognostic scoring system [321]. For the purposes of statistical analysis the aetiological agent was categorised as being either gallstones, alcohol, idiopathic or "other". The patient characteristics are shown in Table 11.

A control population consisting of 102 healthy individuals was derived from donors attending the Lothian plasmaphoresis centre. The control population consisted of 68 males and 34 females, with a median age of 42 years (IQR 36-47). In comparison to patients presenting with acute pancreatitis the control group consisted of significantly fewer females [97/190 (.51) vs 34/102 (.33), χ^2=8.42 p=.004] and was significantly younger [53 (IQR 40-68) vs 42 (IQR 36-47), Mann-Whitney U p<.001)]. Although there were fewer females in the control population, because the tumour necrosis factor and interleukin 1 gene loci are on chromosome 6 and 2 respectively it is unlikely that this difference would introduce bias into the study.

Disease severity [1]	**Mild**	**Severe**	
	n=113	n=77	
Age	51 (38-70)	57 (43-68)	Mann-Whitney U p=.156
Sex	59M:54F	34M:43F	χ^2=1.19, p=.275
Aetiology			
Gallstone	56	28	
Alcohol	31	25	
Idiopathic	15	13	
Other	11	11	χ^2=3.42, p=.331
Glasgow score[321]	1 (0-2) n=111	4 (3-5) n=58	Mann-Whitney U p<.001

Table 11: Characteristics of patients with acute pancreatitis in whom genotype analysis was carried out.

Values are median (interquartile range).

Genotype Analysis

Following informed consent, a 2.5ml sample of peripheral venous blood was collected directly into a blood tube containing ethylenediaminetetraacetic (EDTA) acid (Monovette® Sarstedt, Numbrecht Germany). Samples were stored at -70°C until required at which point they were thawed to room temperature. Extraction and purification of genomic DNA from venous blood was carried out using the Puregene DNA isolation kit (Gentra systems, North Carolina, USA) according to the manufacturer's instructions. The Puregene DNA isolation kit uses a salting out technique [331]. Purified genomic DNA was stored at –20°C.

The distributions of the following four cytokine gene polymorphisms were determined:

TNF-308. A single base G to A substitution may occur at position –308 in the TNFα promoter. This substitution may be identified using polymerase chain reaction gene amplification with subsequent endonuclease digestion [212]. A 107 base pair fragment of the TNFα promoter region from position -331 to -226 was amplified using the primers shown in Table 12. The PCR reaction mixture is given in Table 13. The thermal cycler protocol is given in Table 14. Following gene amplification, PCR products were digested using the *Nco1* endonuclease. The reaction mixture and duration of digestion is shown in Table 15. Digestion products were separated on 9% polyacrylamide gels at a constant voltage of 200V, with subsequent visualisation using ethidium bromide. The cleaved product bands of 87 and 20 base pair lengths represent allele 1 while the uncleaved 107 base pair product represents allele 2 (Figure 3). A permanent record was made using Polapan 665 or 667 Polaroid film.

TNFB. A bi-allelic *Nco1* restriction fragment length polymorphism exists in intron 1 of the tumour necrosis factor β gene. This polymorphism may be identified using polymerase chain reaction gene amplification with subsequent endonuclease digestion. A 368 base pair fragment of the TNFB gene locus was amplified using the primers shown in Table 12. The PCR reaction mixture is given in Table 13. The

thermal cycler protocol is given in Table 14. Following gene amplification, PCR products were digested using the *Nco1* endonuclease. The reaction mixture and duration of digestion is shown in Table 15. Digestion products were separated on 1% agarose gels at a constant voltage of 200V, with subsequent visualisation using ethidium bromide. The cleaved product bands of 586 and 196 base pair lengths represent allele 1 while the uncleaved 782 base pair product represents allele 2 (Figure 4A). A permanent record was made using Polapan 665 or 667 Polaroid film.

Because the primers for the TNFB gene produced other PCR products which could be clearly defined using 6% polyacrylamide gels (Figure 4B), further confirmation that the digestion products were derived from the TNFB gene was required. To demonstrate that the digestion products were derived from the desired TNFB gene locus Southern blot analysis was carried out. A digoxigenin labelled DNA probe (5'-CAGGCAGCAGAACCAGCAGCAGT-3') was constructed. Electrophoretic separation was carried out on 1% agarose gels, which were then neutralised in 0.25M NaCL/0.5M Tris HCl pH7.5 for 1 hour followed by 20xSSC (saline sodium citrate) for 10 minutes. DNA blotting to a nylon membrane was carried out overnight using capillary transfer with 20xSSC. The nylon membrane was exposed to ultraviolet light for 5 minutes in order to achieve fixation of DNA to the membrane. Non-specific DNA probe blocking was minimised by pre-hybridising the membrane in 5xSSC/5xDenhardt's solution/100μg/mL salmon testes DNA for a period of 4 hours. DNA probe hybridisation was carried out using 3.7 nmol/L of labelled probe in 5xSSC/1xDenhardt's solution/100μg/mL salmon testes DNA for 16 hours at a temperature of 60.5°C. The nylon membranes were then buffered in a series of solutions each for a period of 20 minutes (solution 1 - 0.1%SDS/2xSSC : solution 2 - 0.1%SDS/0.2xSSC : solution 3 - 0.1%SDS/0.16xSSC). Detection of the digoxigenin labelled probe was carried out using an alkaline phosphatase conjugated anti-digoxigenin Fab fragment and the chemoluminescent compound CSPD (disodium 3-(4methoxyspirofl,2-dioxetane-3,2'-(5'-chloro) tricyclo [3.3.1.1^{3,7}]decan-4yl0 phenyl phosphate). Probe detection was carried out using a DIG Luminescent Detection Kit from Roche Diagnostics according to the manufacturers instructions. Southern blot confirmed that the PCR bands visualised

following endonuclease digestion of the PCR products were indeed amplified from the TNFB gene (Figure 5). Southern blotting was not carried out for every individual within the study once it was demonstrated that the TNFB digestion products could be consistently and accurately identified.

IL1β. A biallelic *Taq1* restriction fragment length polymorphism exists in exon 5 of the IL1β gene [243]. This polymorphism may be identified using polymerase chain reaction gene amplification with subsequent *Taq1* endonuclease digestion. A 678 base pair fragment of the IL1B gene locus from position +4180 to +4858 was amplified using the primers shown in Table 12. The PCR reaction mixture is given in Table 13. The thermal cycler protocol is given in Table 14. Following gene amplification, PCR products were digested using the *Taq1* endonuclease. The reaction mixture and duration of digestion is shown in Table 15. Digestion products were separated on 6% polyacrylamide gels at a constant voltage of 200V, with subsequent visualisation using ethidium bromide. The cleaved product bands of 156 and 522 base pair lengths represent allele 1 while the uncleaved 678 base pair product represents allele 2 (Figure 6). A permanent record was made using Polapan 665 or 667 Polaroid film.

IL1RN. An 86 base pair variable number tandem repeat sequence exists in intron 2 of the interleukin 1 receptor antagonist gene (IL1RN) [249]. Previous studies have identified the potential for 5 different alleles. This polymorphism may be identified using polymerase chain reaction gene amplification of the IL1RN gene locus between +1990 and +2156 which spans the tandem repeat sequence. The DNA primers used are shown in Table 12. The PCR reaction mixture is given in Table 13. The thermal cycler protocol is given in Table 14. PCR products were separated on 6% polyacrylamide gels at a constant voltage of 200V, with subsequent visualisation using ethidium bromide. PCR product bands of various lengths were identified and correspond to the following alleles; 412 base pair (allele 1), 240 base pair (allele 2), 326 base pair (allele 3), 426 base pair (allele 4), 584 (allele 5) (Figure 7). A permanent record was made using Polapan 665 or 667 Polaroid film.

Polymorphism	Primer sequence 5'→3'		Product length
	Upstream	Downstream	
TNF-308	AGGCAATAGGTTTTGAGG GCCAT	TCCTCCCTGCTCCGATTCC G	107 base pair
TNFB	CCGTGCTTCGTGCTTTGGA CTA	AGAGGGGTGCATGCTTGG GTTC	782 base pair
IL1β	TGTTCTTAGCCACCCCACT C	ATCGCTCCAGCACTCTTGT T	678 base pair
IL1RN	CTCAGCAACACTCCTAT	TCCTGGTCTGCAGGTAA	86 base pair repeat

Table 12: PCR primer sequences for TNF-308, TNFB, IL1β and IL1RN polymorphisms.

	$MgCl_2$ 25mM	PCR buffer 10x	dNTP 2mM	3'primer 2μM	5'primer 2μM	cDNA	Taq polymerase 5U/μL	H_20
TNFB	3μL	5μL	5μL	3μL	3μL	2μL	0.2μL	28.8μL
TNF-308	5μL	5μL	5μL	5μL	5μL	2μL	0.2μL	22.8μL
IL1β	5μL	5μL	5μL	2.5μL	2.5μL	2μL	0.2μL	27.8μL
ILIRN	5μL	5μL	5μL	5μL	5μL	2μL	0.2μL	22.8μL

Table 13: PCR reaction mixtures for TNF-308, TNFB, IL1β and IL1RN polymorphisms.

- $MgCl_2$. Magnesium chloride solution 25mM.
- PCR buffer 10x concentrate. Contains 100mM Tris-HCl (pH9.0 at 25°C), 500mM KCl and 1% Triton X-100.
- dNTP. Mixture of dATP, dTTP, dCTP, dGTP at a concentration of 2mM with respect to each primer.
- 3'primer diluted in sterile water to a concentration of 2μM.
- 5'primer diluted in sterile water to a concentration of 2μM.
- cDNA. Purified cellular DNA.
- Taq polymerase 5units/μL. Stored in 50mM Tris-HCl (pH8.0 at 25°C), 100mM $NaCl_2$, 0.1mM EDTA, 1mM DTT, 50% glycerol, 1% Triton X-100

	Denaturing	Elongation	Completion
TNF-308	94°C 3min, 60°C 1min, 72°C 1min	94°C 1min, 60°C 1min, 72°C 1min x35 cycles	94°C 1min, 60°C 1min, 72°C 5min
TNFB	94°C 1min	94°C 1min, 56°C 1min, 72°C 1min x35 cycles	72°C 10min
IL1β	94°C 10min	94°C 1min, 56°C 1min, 72°C 1min x35 cycles	72°C 10min
IL1RN	94°C 10min	94°C 1min, 60°C 1min, 72°C 1min x35 cycles	72°C 5min

Table 14: PCR cycle regimes for TNF-308, TNFB, IL1β and IL1RN polymorphisms.

Endonuclease	PCR product	Buffer 10x	Enzyme	Distilled Water	Digestion
Nco1	15µL	5µL	0.5µL	29.5µL	37°C 4 hours
Taq1	15µL	5µL	1µL	29µL	65°C 4 hours

Table 15: Endonuclease digestion.

Table demonstrated constituents of reaction mixture and conditions of digestion.

- *Nco1* 10x buffer contains 100mM Tris-HCl, 1000mM NaCl, 100mM $MgCl_2$ and 100mM 2-mercaptoethanol.
- *Taq1* 10x buffer contains 330mM Tris-acetate, 660 mM K-acetate, 100mM Mg-acetate and 5mM dithiothreitol

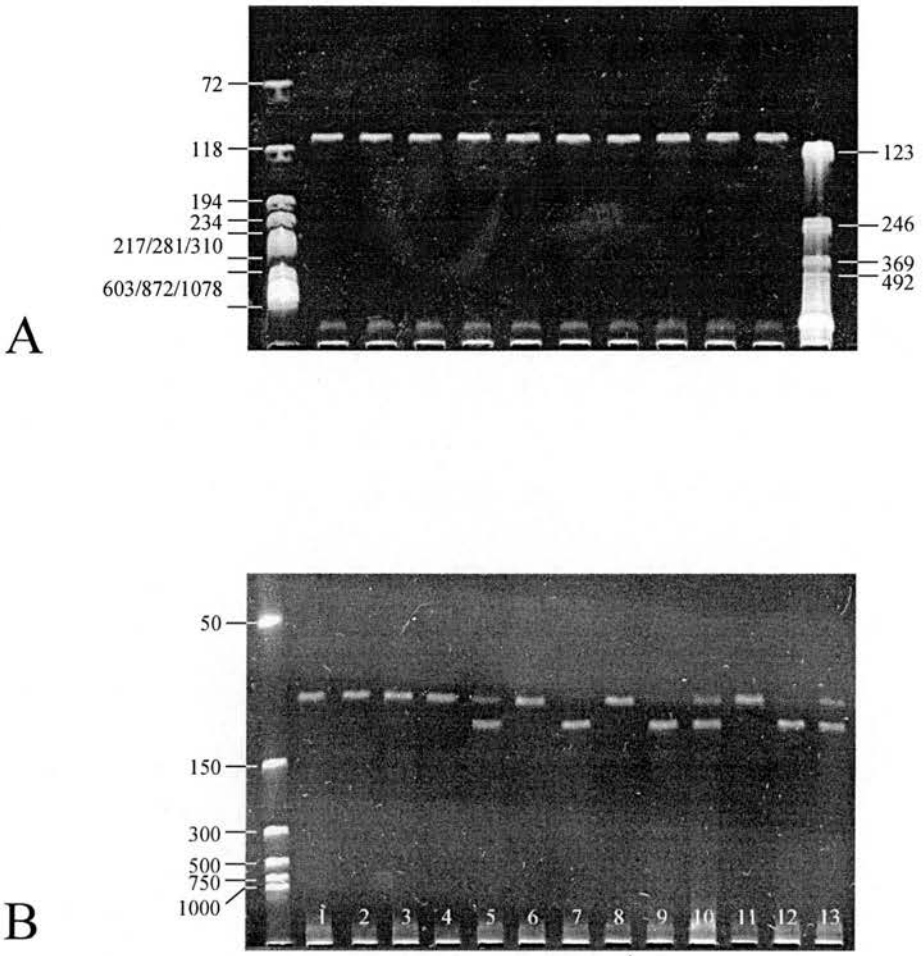


Figure 3: TNF –308 Gene Polymorphism.

A) PCR with primers designed to include position –308 of the TNFα gene. PCR product of 107 base pair length. Electrophoresis on 9% polyacrylamide gel.

B) *Nco1* endonuclease digestion of TNF-308 PCR product demonstrating digestion fragments of 107 and 87 base pair length. Digestion product of 20 base pair length is too small to be displayed on the gel. Lanes 1, 2, 3, 4, 6, 8 and 11 homozygous allele 1; lanes 5, 10 and 13 heterozygous allele 1 and 2; lanes 7, 9 and 12 homozygous allele 2. Electrophoresis on 9% polyacrylamide gel.

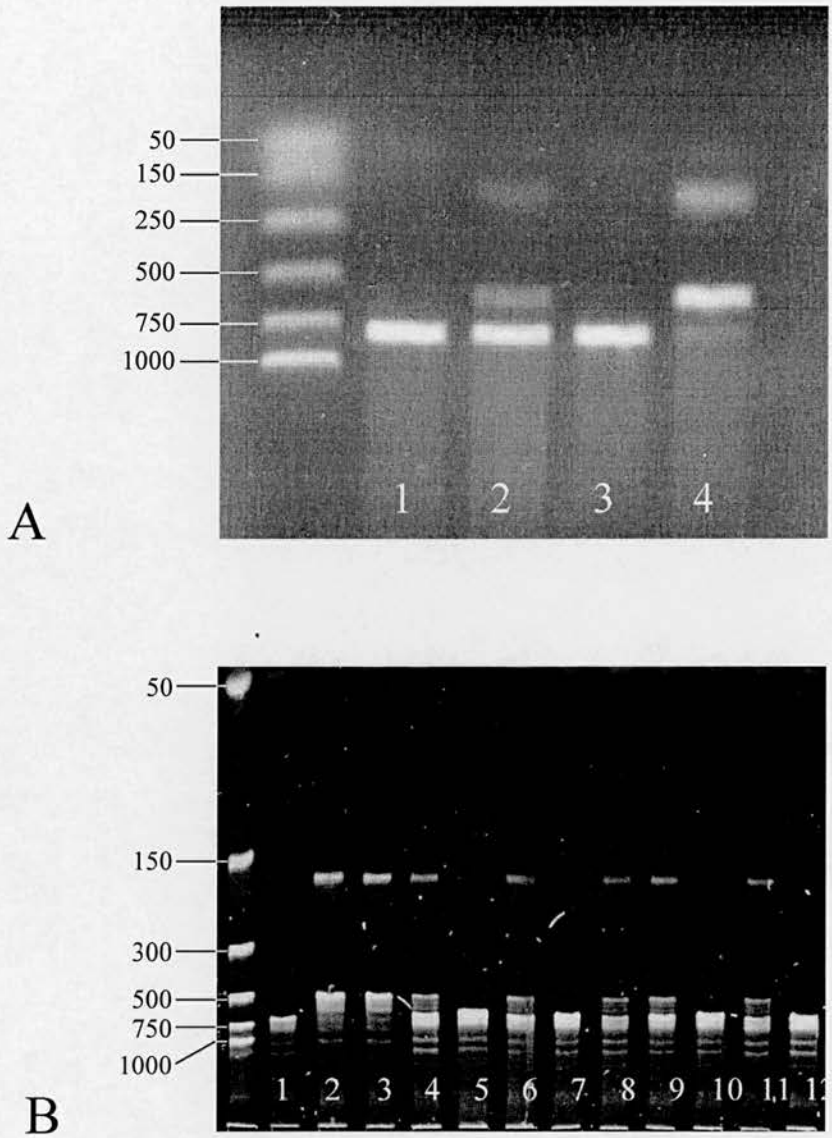
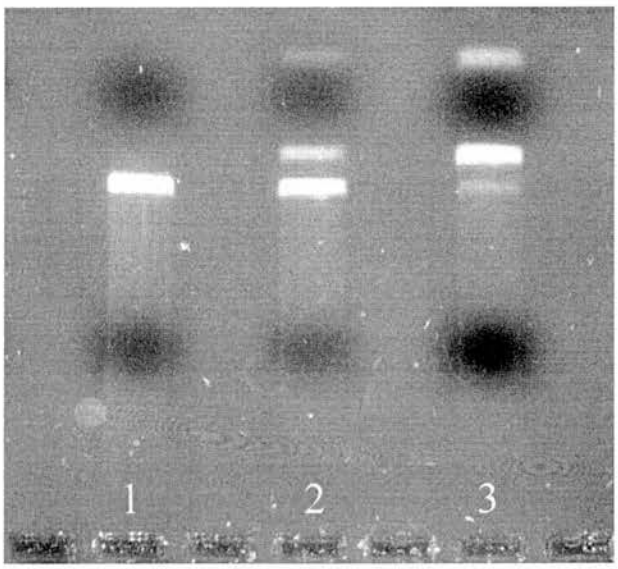


Figure 4: TNFB gene polymorphism.

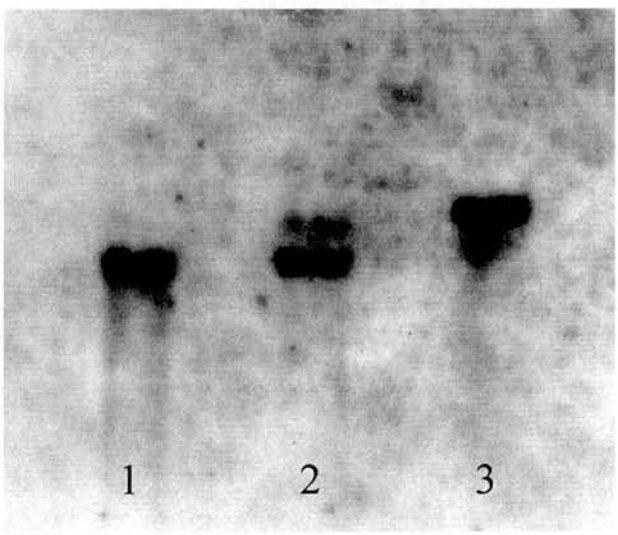
PCR with TNFB primers followed by *Nco1* endonuclease digestion. Product band lengths correspond to the following alleles: allele 1 - 586 and 196 base pair ; allele 2- 782 base pair.

A) PCR with TNFB primers followed by Nco1 endonuclease digestion. Electrophoresis on 1% agarose gel. Lane 1 homozygous allele 2; lane 2 heterozygous allele 1 and 2; lane 3 homozygous allele 2; lane 4 homozygous allele 1.

B) PCR with TNFB primers followed by Nco1 endonuclease digestion. Electrophoresis on 6% polyacrylamide gel. Lanes 1, 5, 7, 10 and 12 homozygous allele 2; lanes 4, 6, 8, 9 and 11 heterozygous allele 1 and 2; lanes 2 and 3 homozygous allele 1. Note additional bands clearly visible on polyacrylamide gel but not on agarose gel.



A



B

Figure 5: Southern blot for TNFB gene.

A) TNFB genotype on 1% agarose gel. Lane 1 homozygous allele 2; lane 2 heterozygous allele 1 and 2; lane 3 homozygous allele 1.

B) Southern blot using labelled probe directed at TNFB genotype PCR product. Lane 1 homozygous allele 2; lane 2 heterozygous allele 1 and 2; lane 3 homozygous allele 1.

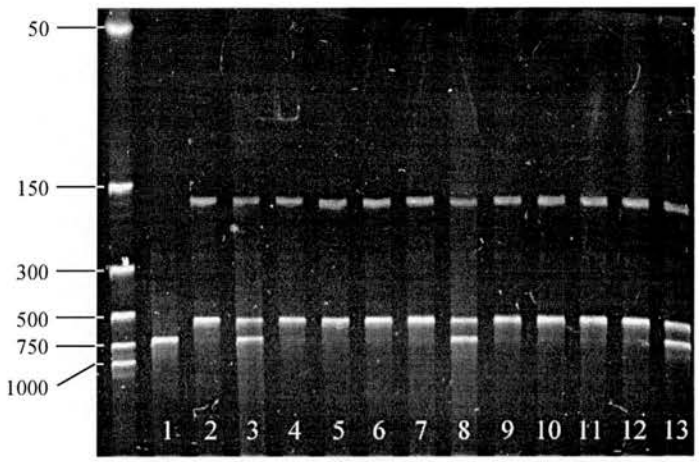


Figure 6: IL1β gene polymorphism.

PCR with IL1B primers followed by *Taq1* endonuclease digestion. Electrophoresis on 6% polyacrylamide gel. Product band lengths correspond to the following alleles: allele 1 - 156 and 522 base pair ; allele 2- 678 base pair. Lane 1 homozygous allele 2; lanes 3, 8 and 13 heterozygous allele 1 and 2; lanes 2, 4, 5, 6, 7, 9, 10, 11 and 12 homozygous allele 1.

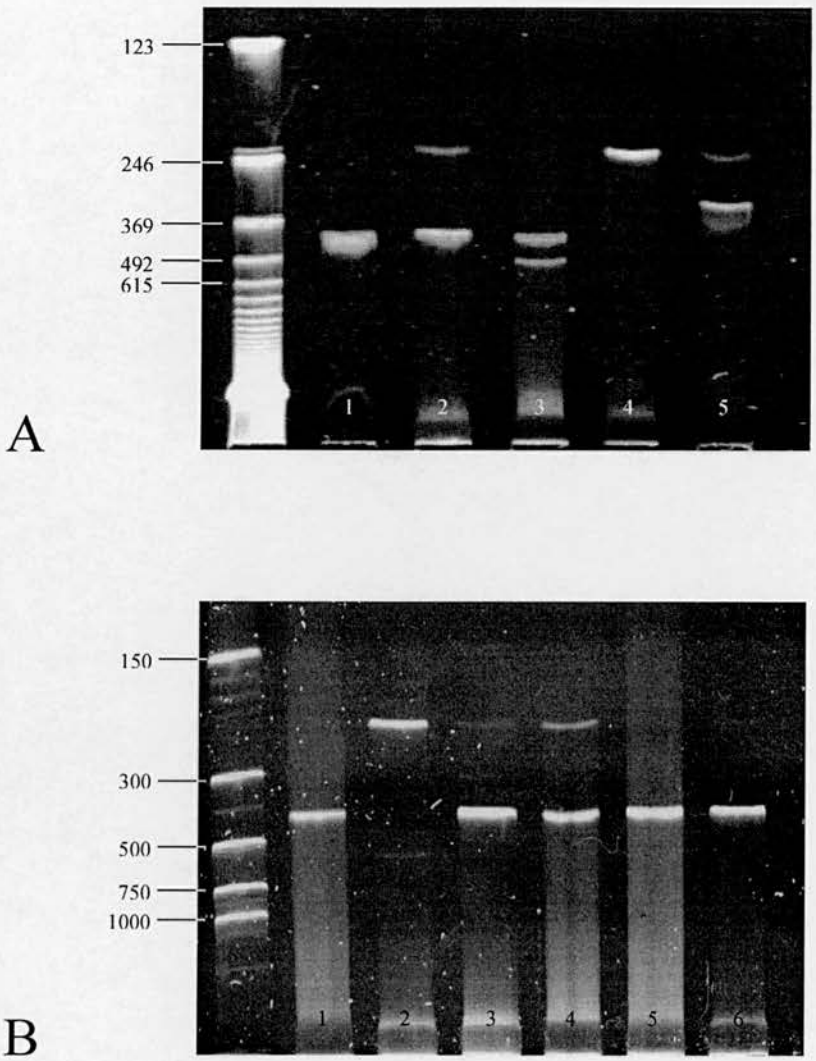


Figure 7: IL1RN gene polymorphism.

PCR with IL1RN primers to detect the variable number tandem repeat sequence within the IL1RA gene. PCR product length corresponds to the following alleles: 412 base pair (allele 1), 240 base pair (allele 2), 326 base pair (allele 3), 426 base pair (allele 4), 584 base pair (allele 5). Electrophoresis on 6% polyacrylamide gel.

A) PCR with IL1RN primers. Lane 1 homozygous allele 1; lane 2 heterozygous allele 1 and 2; lane 3 heterozygous allele 1 and 4; lane 4 homozygous allele 2; lane 5 heterozygous allele 2 and 3.
B) PCR with IL1RN primers. Lanes 1, 3, 5 and 6 homozygous allele 1; lane 2 homozygous allele 2; lane 4 heterozygous allele 1 and 2.

Statistical Analysis

Comparison of allelic and genotypic frequencies was carried out using the χ^2 test. Continuous variables are presented as median and interquartile range. Continuous variables were compared using the non-parametric Mann-Whitney U, Wilcoxon signed rank and Kruskal-Wallis tests thereby avoiding any distributional assumptions. Statistical significance was taken at the p=.05 level. The relationship between the carriage of individual alleles and disease severity is presented as odds ratio with 95% confidence interval (OR 95%C.I.). Statistical analysis was carried out using the Statview 5.0 software package (SAS Institute, North Carolina, USA).

Ethical Approval

This study was approved by the Lothian ethics committee. Informed consent was obtained from all participants or from the next of kin if the participant was unable to give consent.

Study Population

Because down-regulation of leukocyte function may occur during the systemic inflammatory response syndrome [332], patterns of cytokine secretion were determined following recovery from the episode of acute pancreatitis. Surviving patients who had previously consented to the genotype studies were invited by letter to the out-patient clinic with the intention of studying patterns of cytokine secretion. Patients were deemed to have recovered from their acute pancreatitis if they had no on-going symptoms related to their acute pancreatitis, nor an on-going complication. Patients were excluded from study if they were taking either non-steroidal anti-inflammatory drugs (NSAIDs) or immunosuppressive therapy. In total 57 patients responded to the request, however 6 patients were ineligible for study because they were currently taking NSAIDs or corticosteroids. A total of 51 patients were therefore eligible for phenotype analysis. The characteristics of this subgroup are shown in Table 16. There were no significant differences with respect to age (Mann-Whitney U p=.276), sex (χ^2=0.10, p=.752), aetiology (χ^2=5.12, p=.160), Glasgow score (Mann-Whitney U p=.119) and end of episode disease severity (χ^2=2.09, p=.149) between those individuals who did or did not undergo post-episode cytokine analysis (Table 17). The median interval between discharge from hospital discharge to determination of cytokine phenotype was 287 days (IQR 189.5-465.5). Further, in order to quantify any inflammatory response still present, serum C-reactive protein concentrations were assayed. For the 51 patients studied the median C-reactive protein concentration was <1.0g/dL (IQR <1.0-1.9 g/dL).

As would be expected those with severe acute pancreatitis had significantly higher Glasgow scores than those with previous mild disease (Mann-Whitney U p<.001). Interestingly, there was a significant difference in the aetiological agent inducing acute pancreatitis between the two groups (χ^2=8.31, p=.040). Although the majority of cases of severe acute pancreatitis were due gallstones and alcohol excess, in 6

individuals the aetiological agents were post-endoscopic retrograde cholangio-pancreatography (2), hyperlipidaemia (1), angiography (1), diabetic ketoacidosis (1) and pancreas divisum (1). All cases of mild acute pancreatitis were due to either gallstones or alcohol, or were idiopathic.

	Mild	Severe	
	n=26	n=25	
Age	58 (45-73)	55 (41-63)	Mann-Whitney U p=.337
Sex	11M:15F	13M:12F	χ^2=0.48, p=.488
Aetiology			
Gallstone	15	11	
Alcohol	4	5	
Idiopathic	7	3	
Other	0	6	χ^2=8.31, p=.040
Glasgow score [321]	2 (1-3) n=25	4 (3-5) n=18	Mann-Whitney U p<.001

Table 16: Characteristics of patients undergoing phenotype analysis.

Patient characteristics for those patients undergoing phenotype analysis following recovery from acute pancreatitis. Stated values are median (interquartile range).

	Phenotyped	Not phenotyped	
	n=51	n=139	
Age	57 (44-70)	51 (39-69)	Mann-Whitney U p=.276
Sex	24M:27F	69M:70F	χ^2=0.10, p=.752
Aetiology			
Gallstone	26	58	
Alcohol	9	47	
Idiopathic	10	18	
Other	6	16	χ^2=5.12, p=.160
Severity [1]	26 mild:25 severe	87 mild:52 severe	χ^2=2.09, p=.149
Glasgow score [321]	3 (1-4) n=43	2 (1-4) n=126	Mann-Whitney U p=.119

Table 17: Characteristics of participants and non-participants of phenotype studies.

Comparison of characteristics of patients who underwent determination of cytokine secretory patterns with those of patients not participating. Stated values are median (interquartile range).

Cytokine Secretion Studies

Cytokine production was determined using an ex-vivo whole blood culture technique [333]. The whole blood technique has previously been demonstrated to be reproducible and accurate in the investigation of genetically determined cytokine levels [334]. In brief 5mL of peripheral venous blood was collected into heparinised blood tubes (Monovette® Sarstedt, Germany). A total of 2mL heparinised venous blood was diluted 1 in 10 with RPMI culture medium (Life Technologies, Paisley UK) supplemented with penicillin 50units/mL, streptomycin 50μg/mL and glutamine 2mmol/L. Aliquots of 2mL were cultured in 16 well culture plates (Corning Costar, High Wycombe UK) at 37°C, humidified with 5% CO_2. Paired samples were stimulated with lipopolysaccharide (LPS) derived from Escherichia coli 0127:B8 (Sigma, Poole UK). LPS was chosen as the experimental stimulus because previous studies have suggested that endotoxin has a major role in promoting the inflammatory response in human acute pancreatitis [92, 96, 318, 319]. Following incubation samples were removed from the culture plate and centrifuged at 450G for 5 minutes. The supernatants were stored at -70°C until measurement of cytokine concentration.

Culture supernatant cytokine concentrations were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits: TNFα (CLB, Amsterdam The Netherlands), IL1β (R&D Systems Europe, Abingdon UK) and IL1RA (R&D Systems Europe, Abingdon UK). Culture supernatant cytokine concentrations were determined for paired samples. The lower limits of detection were as follows: TNFα 14pg/mL, IL1β 20 pg/mL, IL1RA 285pg/mL. The intra-assay and inter-assay coefficients of variation for each assay were: TNFα 14.9% and 8.9%, IL1β 4.2% and 8.3%, IL1RA 3.8% and 15.6%.

Peripheral venous blood was also collected at the same time as blood for whole blood culture for the determination of haematological indices. A total of 2.5mL venous blood was collected into tubes containing EDTA. Haemoglobin

concentration and white blood count and differential were carried out on an automated analyser (Sysmey NE8000, Toa Medical Electronics, Japan).

Experimental Conditions For Determining Cytokine Phenotype

In order to determine the optimum concentration of LPS and duration of incubation for determining patterns of cytokine secretion, a series of trial experiments was carried out.

In the first experiment venous blood was obtained from 7 unrelated healthy individuals. Whole blood cultures were stimulated with increasing concentrations of lipopolysaccharide; 0μg/mL, 5μg/mL and 10 μg/mL. Cultures were then incubated for either 8 or 24 hours. The TNFα supernatant concentrations are shown in Figure 8. After 8 hours incubation, when compared to the unstimulated sample TNFα concentration was significantly higher following stimulation with 5μg/mL LPS (p=.028, Wilcoxon signed rank) and 10μg/mL LPS (p=.028, Wilcoxon signed rank), however there was no significant difference in TNFα secretion between the samples stimulated with 5μg/mL LPS or 10μg/mL LPS (p=.249, Wilcoxon signed rank). Similarly following 24 hours incubation, when compared to the unstimulated sample, TNFα concentration was significantly higher following stimulation with 5μg/mL LPS (p=.018, Wilcoxon signed rank) and 10μg/mL LPS (p=.018, Wilcoxon signed rank). Again there was no significant difference in TNFα secretion between the samples stimulated with 5μg/mL LPS or 10μg/mL LPS (p=.398, Wilcoxon signed rank). Interestingly, supernatant TNFα concentrations were significantly higher after 8 hours than 24 hours incubation following stimulation with either 5μg/mL LPS (p=.028, Wilcoxon signed rank) or 10μg/mL LPS (p=.028, Wilcoxon signed rank).

In order to further define an optimum duration of culture, paired samples derived from 7 unrelated healthy individuals were stimulated with LPS at a final concentration of 5μg/mL or were left unstimulated. Samples were incubated for a

period of 1, 4 or 8 hours. The results are shown in Figure 9. At all time points the concentration of TNFα was significantly higher in the stimulated sample when compared to the unstimulated sample (1 hour p=.0180 Wilcoxon signed rank, 4 hours p=.0180 Wilcoxon signed rank and 8 hours p=.0277 Wilcoxon signed rank). Further, there was a significant increase in TNFα concentrations in the stimulated samples over the study period (ANOVA p<.001). Similarly, although the rise over time was small, TNFα concentrations increased significantly in the unstimulated samples over the study period indicating spontaneous secretion of TNFα (ANOVA p=.007).

Following these experiments a decision was made that for the cytokine secretory phenotype studies paired samples were to be cultured with or without LPS at a final concentration of 5μg/mL and incubated for a period of 8 hours.

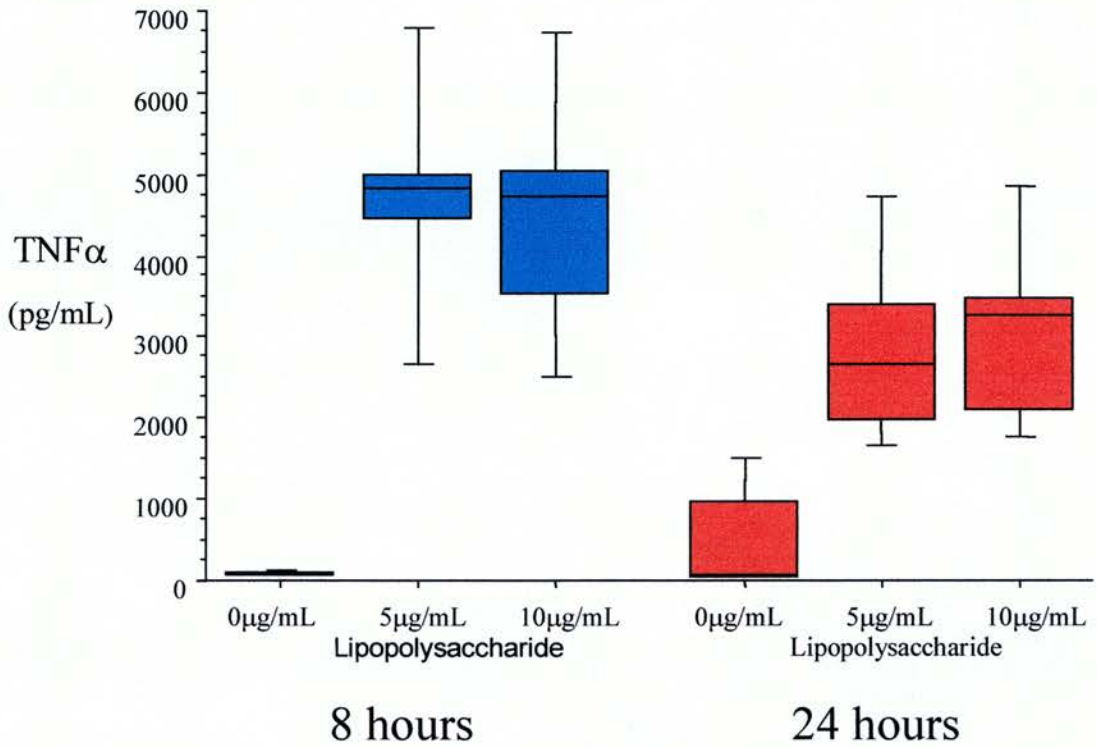


Figure 8: Whole blood TNFα secretion following lipopolysaccharide stimulation at differing concentrations.

Box plot of whole blood supernatant TNFα concentrations following stimulation with lipopolysaccharide; 0μg/mL, 5μg/mL and 10 μg/mL. Whole blood cultures were incubated for either 8 or 24 hours. Venous blood was obtained from 7 unrelated healthy individuals. Box plot illustrates median and 10th, 25th ,75th and 90th centiles.

8 hours incubation

LPS 0μg/mL vs LPS 5μg/mL	p=.028, Wilcoxon signed rank
LPS 0μg/mL vs LPS 10μg/mL	p=.028, Wilcoxon signed rank
LPS 5μg/mL vs LPS 10μg/mL	p=.249, Wilcoxon signed rank

24 hours incubation

LPS 0μg/mL vs LPS 5μg/mL	p=.018, Wilcoxon signed rank
LPS 0μg/mL vs LPS 10μg/mL	p=.018, Wilcoxon signed rank
LPS 5μg/mL vs LPS 10μg/mL	p=.398, Wilcoxon signed rank

LPS 5μg/mL

8 hours vs 24 hours p=.028, Wilcoxon signed rank

LPS 10μg/mL

8 hours vs 24 hours p=.028, Wilcoxon signed rank

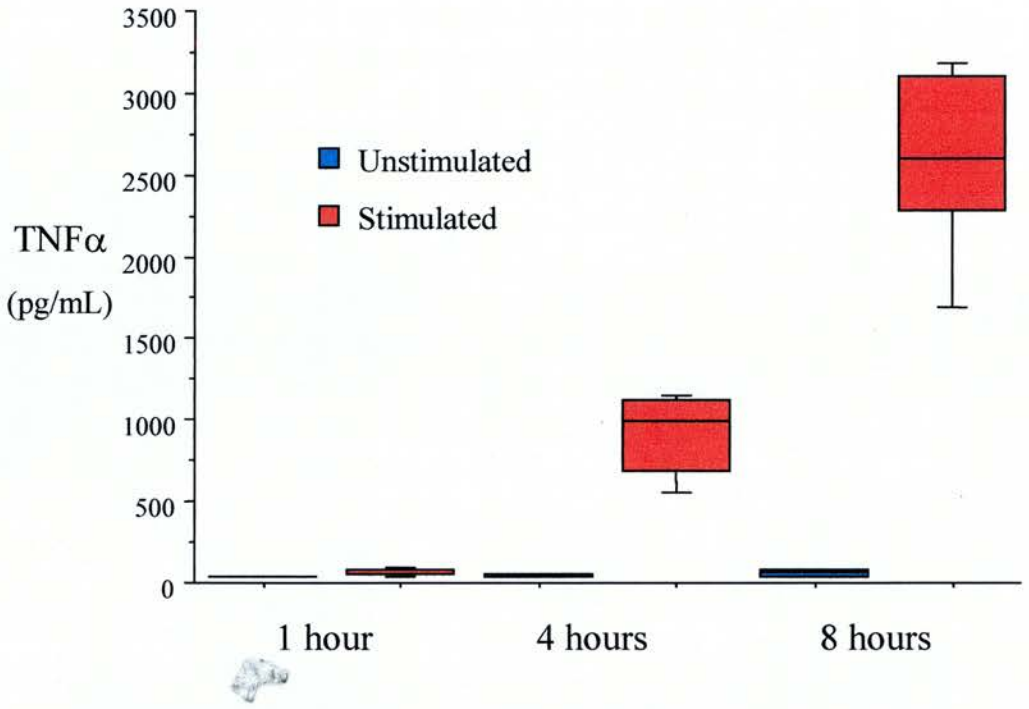


Figure 9: Whole blood TNFα secretion following lipopolysaccharide stimulation at differing time points.

Box plot of whole blood supernatant TNFα production over an 8 hour period. Venous blood was obtained from 7 unrelated healthy individuals. Paired samples were incubated with stimulation using LPS at a final concentration of 5μg/mL or without stimulation. Whole blood cultures were incubated for 1, 4 or 8 hours. At all time points TNFα concentration was significantly higher in the stimulated samples when compared to the unstimulated sample (1 hour p=.0180, Wilcoxon signed rank, 4 hours p=.0180, Wilcoxon signed rank and 8 hours p=.0277, Wilcoxon signed rank). In both stimulated and unstimulated samples the concentration of TNFα increased over the study period (unstimulated ANOVA p=.007, stimulated p<.001). Box plot illustrates median and 10^{th}, 25^{th} ,75^{th} and 90^{th} centiles.

Statistical Analysis

Continuous variables are presented as median and interquartile range. Continuous variables were compared using the non-parametric Mann-Whitney U, Wilcoxon signed rank and Kruskal-Wallis tests thereby avoiding any distributional assumptions. However, for the experiments using repeated measures over time an analysis of variance (ANOVA) technique was also used. Statistical significance was taken at the p=.05 level. Statistical analysis was carried out using the Statview 5.0 software package (SAS Institute, North Carolina, USA).

Ethical Approval

This study was approved by the Lothian regional ethics committee. Informed consent was obtained from all participants.

Patients

Patients admitted with prognostically severe acute pancreatitis within 72 hours of the onset of symptoms were eligible for study. The diagnosis of acute pancreatitis was based on a history consistent with acute pancreatitis and a serum amylase concentration greater than three times the upper limit of normal or radiological signs of acute pancreatitis at computed tomography. Prognostication of disease severity was carried out using the Glasgow [321] and APACHE II [329] scoring systems. Patients were considered to have prognostically severe acute pancreatitis if they had a Glasgow score ≥ 3 and, or, an APACHE II score ≥ 7. Patients were excluded from study if their age was less than 18 or greater than 80, if they were pregnant, if they had previously received nutritional support or if they were enrolled in another clinical trial.

During the period 16th December 1996 to 1st July 1998, 156 patients were admitted to the Royal Infirmary of Edinburgh with a diagnosis of acute pancreatitis. Of those patients 51 were eligible for inclusion in the study, however 17 refused entry, 3 patients were entered into another trial, 1 patient was commenced on normal diet within 24 hours of admission, in 1 patient the diagnosis was initially missed and 1 patient succumbed to their disease before they could be entered into the trial. A total of 28 patients met the entry criteria and were randomised. One patient who met all of the entry criteria was subsequently found at laparotomy to have an acute mesenteric infarction and was therefore withdrawn from the trial. The results of this individual have been excluded from analysis.

Following informed written consent patients were randomised in blocks of variable even numbers to receive either conventional therapy plus early enteral nutrition or conventional therapy alone. Randomisation in blocks was employed to reduce the potential for clustering in one or other arm within in a small study population. The

individual responsible for drawing the randomisation card was unaware of the number of patients in each randomisation block. Patients with an APACHE II score of >20 were stratified and randomised separately to minimise the potential for clustering of patients with very severe disease in one treatment arm.

To further define the severity of the acute pancreatitis at admission to the study, a contrast-enhanced computed tomography was carried out on each patient as soon as was practicable allowing calculation of the Balthazar [322] and Helsinki [335] prognostic scores, both of which have been validated in the determination of severe disease.

For the purpose of statistical analysis, the aetiological agent was categorised as either gallstone, alcohol, idiopathic or other.

The demographics of the study sample are shown in Table 18.

	Enteral nutrition	Conventional therapy	
	n=13	n=14	
Sex	5M:8F	9M:5F	χ^2=1.80 p=.180
Age	64 (26-76)	51.5 (23-78)	Mann-Whitney U p=.423
Aetiology			
Gallstone	7	3	
Alcohol	4	7	
Idiopathic	1	2	
Other	1	2	χ^2=3.05 p=.384
Glasgow score [321]	4 (1-5)	3.5 (1-6)	Mann-Whitney U p=.884
APACHE II score [329]	10 (7-20)	11.5 (7-32)	Mann-Whitney U p=.560
Helsinki score [335]	4 (1-6)	4 (0-7)	Mann-Whitney U p=.716
Balthazar score [322]	3 (0-8)	2.5 (0-8)	Mann-Whitney U p=.942
Duration of symptoms prior to admission to study (hours)	31 (10-72)	27 (6-68)	Mann-Whitney U p=.254
Duration of hospital stay (days)	10 (5-30)	9.5 (5-94)	Mann-Whitney U p=.716
End of episode Atlanta category [1]	6 mild : 7 severe	5 mild : 9 severe	χ^2=0.30 p=.581

Table 18: Demographics of patients randomised in enteral nutrition trial.

Stated values are median (range).

Study Endpoints

The primary endpoint of this study was modulation of markers of the inflammatory response in patients with predicted severe acute pancreatitis. As measures of the systemic inflammatory response the following were assayed: serum C-reactive protein (CRP), serum interleukin 6 (IL6) and serum soluble TNF receptor I (sTNFR-I). Levels of serum IL6 and sTNFR-I have previously been demonstrated to increase early in patients with acute pancreatitis and to correlate with disease severity [91]. Serum CRP concentrations have also been demonstrated to correlate with disease severity but peak levels occur 24-48 hours after the peak for IL6 concentrations [143]. As an indirect measure of exposure to endotoxin, serum anti-endotoxin core IgG antibody concentrations were assayed. Previous evidence indicates that endotoxin may be a potent stimulus for the systemic inflammatory response in severe acute pancreatitis and that endotoxin exposure can be a consequence of intestinal dysfunction [92, 317, 318]. Levels of serum IgG anti-endotoxin core antibody have been demonstrated to reflect previous endotoxin exposure and can aid in the identification of patients with acute pancreatitis at risk of developing organ dysfunction [319].

The secondary endpoints were the safety and tolerance of early enteral nutrition, markers of intestinal dysfunction and organ dysfunction scores.

i. *Safety And Tolerance Of Early Enteral Nutrition*: For each 24 hour period the volume of feed delivered was measured. All adverse events that were potentially attributable to enteral nutrition were recorded. A simple graded nausea score (0= no nausea, 1= nausea present, 2= vomiting) was used and patients were assessed daily to quantify nausea. In order to assess the potential of early enteral nutrition to modify the catabolic processes found in severe acute pancreatitis, urinary nitrogen excretion was calculated for each 24 hour period.
ii. *Modulation Of Intestinal Dysfunction*: Intestinal permeability was determined using a differential sugar permeability test [266, 267]. The urinary excretion of the orally administered non-absorbable probes L-rhamnose (monosaccharide) and lactulose (disaccharide) was measured to determine intestinal permeability.

iii. *Organ Dysfunction*: Organ dysfunction was quantified using the Marshall organ dysfunction scoring system [336]. The Marshall organ dysfunction score summates individual scores for cardiovascular, respiratory, renal, haematological, neurological and hepatic function giving rise to a score between 0 and 24 inclusive.

Study Period

The patient was deemed to have completed the study following the introduction of normal diet, the wish to be removed from the trial or upon completion of the tenth day.

Conventional Therapy

Conventional therapy was in accordance with current British Society of Gastroenterology guidelines for the management of patients with acute pancreatitis [14]. This consisted of a nil-by-mouth regime, fluid resuscitation, oxygen therapy as required, urethral catheterisation with hourly monitoring of urine volume and opiate analgesia. Central venous cannulation was carried out at the discretion of the treating physician. Metabolic abnormalities were corrected when present. Organ support was carried out as required. In light of recent randomised controlled trials demonstrating the benefit of prophylactic antibiotic therapy in patients with severe acute pancreatitis [337], all patients were prescribed intravenous cefotaxime 2g and metronidazole 500mg three times daily for 4 days. In patients with a history of hypersensitivity to cephalosporins or penicillins, ciprofloxacin was prescribed in place of cefotaxime. After 4 days all antibiotic therapy was stopped, with further antibiotic therapy being prescribed according to antibiotic sensitivities of positive bacteriological cultures, or empirically at the discretion of the treating clinician.

All patients had contrast-enhanced computed tomography. Radiological severity of acute pancreatitis was quantified using the Balthazar [322] and Helsinki [335] scoring

systems. In addition patients without evidence of gallstones on CT underwent trans-abdominal ultrasonography to determine the presence or absence of gallstones.

In line with current evidence, early ERCP was only carried out in patients with evidence of acute cholangitis or in patients with obstructive jaundice and a worsening clinical condition [338].

Nasojejunal Feeding

Single lumen 110 cm 8 French (Corsafe® Merck, Middlesex UK) nasojejunal feeding tubes were placed under flouroscopic screening such that the tip of the nasojejunal feeding tube was distal to the ligament of Treitz. The duration of radiological screening taken to achieve the correct tube position was recorded. Tube dislodgement episodes were defined as a loss of tube position such that the tip of the nasojejunal feeding tube was no longer distal to the ligament of Trietz. Routine radiological assessment of the position of the tip of the feeding tube following the initial radiological screening procedure was not carried out. Enteral nutrition was commenced at a rate of 25mL/hr, increasing daily by 25mL/hr until the desired caloric intake, as determined by the Schofield equation [339], was reached. Nasojejunal tubes were initially aspirated 4 hourly to exclude pooling of feed. In the presence of signs of feed intolerance (increasing abdominal distension, regurgitation, high nasojejunal tube aspirates) the infusion rate was not increased.

Although studies exist suggesting maximal benefit from the use of an immune-enhancing nutrition supplement [290, 291], these data had not been reported at the time of the inception of the present study, and therefore a standard nutritional formulation was used. Jevity® (Abbott, Berkshire UK), a polymeric formula containing fibre, was used in all cases. 500 ml Jevity contains 4g protein, 3.5g fat, 13.1g carbohydrate and 1.4g dietary fibre providing 2105 kilojoules.

Laboratory Tests

Venous blood sampling was carried out at 0800 each day. Blood samples were centrifuged at 900G for 10 minutes. Serum was then removed and stored at -70°C until analysis.

Serum CRP was assayed at admission and on days 2,4,7 and 10 using an automated fluorometric immunoassay technique (Abbott, Berkshire UK). The lower limit of detection was 0.1mg/dL. The inter-assay coefficient of variation was less than 10%.

Serum IL6 was measured on admission and on days 2 and 4 using an enzyme-linked immunoassay technique (ELISA) (CLB, Amsterdam The Netherlands). The lower limit of detection was 33pg/mL. The intra-assay and inter-assay coefficients of variation were 1.6% and 4.0% respectively.

Serum sTNFR-I was measured at admission and on days 2,4,7 and 10 using an ELISA technique (R&D Systems, Abingdon UK). The lower limit of detection was 156pg/mL. The intra-assay and inter-assay coefficients of variation were 7.1% and 7.6% respectively.

Total urinary nitrogen excretion was calculated through the measurement of urinary urea. Urinary urea concentration was determined using an automated analyser and was carried out by Quintiles (Edinburgh UK). From this the total urinary nitrogen excretion was calculated, following the assumption that urinary urea nitrogen accounts for only 80% of urine nitrogen loss.

Serum anti-endotoxin core antibody concentration were assayed using an ELISA technique (Coaset Endocab Chromogenix, Mölndal Sweden). The lower limit of detection was 6MU/mL. The intra-assay and inter-assay coefficients of variation were 14.2% and 9.7% respectively.

Urinary excretion of non-absorbable sugars was measured on admission and on days 4 and 10. Following a period of fasting of no less than 8 hours, a solution containing lactulose 5g, L-rhamnose 1g, sucrose 20g and lactose 20g (Ninewells Pharmaceuticals, Dundee UK) dissolved in 80 ml water was administered orally. Lactose and sucrose were used to increase the osmolality of the sugar solution, thereby increasing the sensitivity of the test. Following administration urine was collected for the next 5 hours. Urine sugar concentration was determined by high performance liquid chromatography within the Department of Gastroenterology, Western General Hospital, Edinburgh. Urinary lactulose/rhamnose (L/R) ratio was calculated and compared to a previously determined normal laboratory reference range. Increased L/R ratio is indicative of small intestine injury, whilst the absorption of sucrose indicates gastric injury. The disaccharide peak for lactulose could not be quantified if other disaccharides were present. Further, excessive glycosuria prevented L-rhamnose measurement. Intestinal permeability measurements were categorised as "normal", "increased lactose/rhamnose ratio" and "sucrose permeability". Samples with glucose present were excluded from statistical analysis.

Sample Size Calculations

A previous study in which 34 patients with acute pancreatitis were randomised to receive either early enteral or parenteral nutrition reported a 64% reduction in the systemic inflammatory response syndrome in patients with acute pancreatitis receiving early enteral nutrition [326]. This study also reported a 54% reduction in CRP in this group. There was a 77% difference in the cumulative incidence of sepsis, multiple organ dysfunction, need for operative intervention and mortality between patients receiving early enteral nutrition and those in the control arm. On this basis a 50% reduction in the markers of the inflammatory response used in this study would be detected with 80% power, (α=0.05) with 12 patients in each arm.

Statistical Analysis

In the majority of instances stated values are median (range) with statistical analysis being carried out using non-parametric tests to avoid distributional assumptions. However, for the comparison of repeated measurements over the study period mean (standard error) are given and analysis of variance techniques (ANOVA) were used. In all cases a two tailed test was used. Statistical significance was taken at the $p=.05$ level. Statistical analysis was carried out using the Statview 5.0 software package (SAS Institute, North Carolina, USA).

Ethical Approval

This study was approved by the Lothian research ethics committee. Informed consent was obtained from the patient or their next of kin.

Results

Kinetics Of Serum Soluble E-Selectin And P-Selectin In Acute Pancreatitis

Serum Selectin Levels

The pattern of serum soluble E-selectin over the first 3 days of admission to hospital in patients with acute pancreatitis is demonstrated in Figure 10A. There was no significant association between serum soluble E-selectin concentration and the day of sample (ANOVA p=.090). Similarly, no significant association existed between serum soluble E-selectin concentration and disease severity (ANOVA p=.451). However, with respect to serum soluble E-selectin concentration a significant interaction existed between the day of sample and disease severity (ANOVA p=.044). The pattern of increasing serum soluble E-selectin concentrations over the first 3 days in patients with severe disease is significantly different from the pattern observed in patients with mild disease. Similarly serum soluble E-selectin concentrations increased over the first three days of admission in non-survivors, and were significantly different from the pattern observed in survivors (Figure 10B. ANOVA P=.001).

The pattern of serum soluble P-selectin over the first 3 days of admission to hospital in patients with acute pancreatitis is demonstrated in Figure 11A. In patients with mild and severe acute pancreatitis a significant fall in serum soluble P-selectin concentrations was observed (ANOVA p<.001). There was no significant difference in serum soluble P-selectin concentrations between patient groups (ANOVA p=.366). There was also no significant interaction between the day of sample and disease severity in determining serum soluble P-selectin concentrations (ANOVA p=.254). In contrast, with respect to mortality, serum soluble P-selectin concentrations were significantly higher over the first three days of admission in non-survivors than in survivors (Figure 11B. ANOVA P=.005), primarily on day 1.

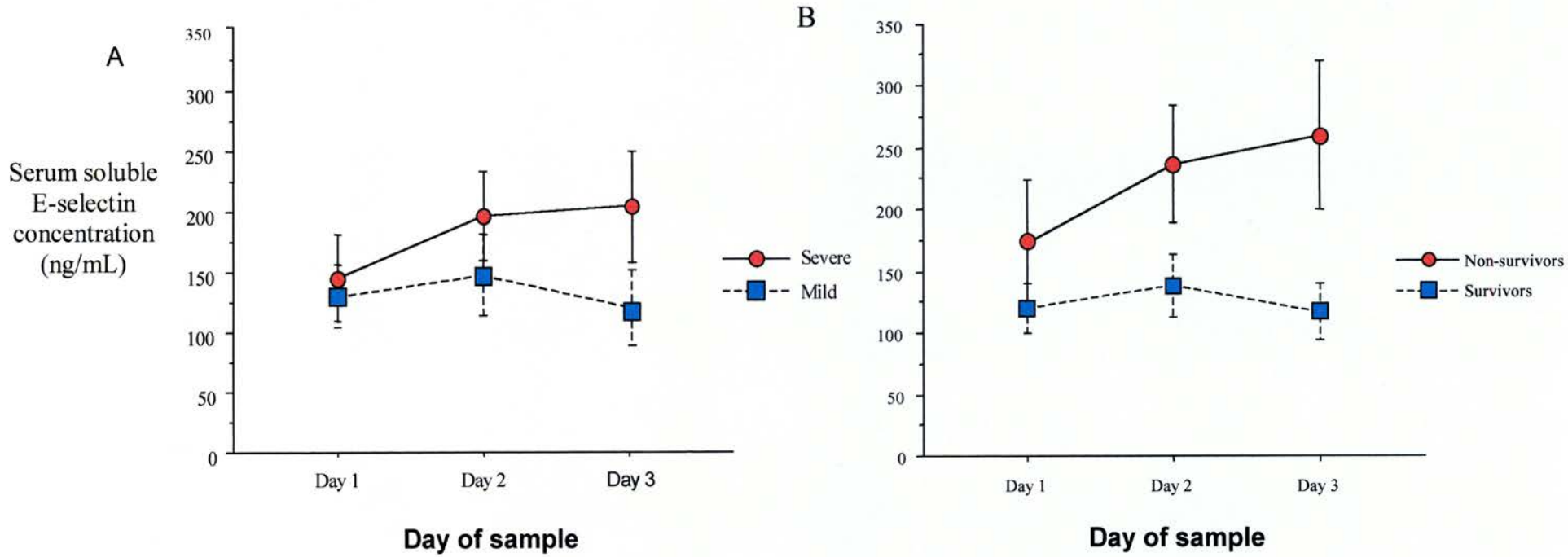


Figure 10: Serum soluble E-selectin concentrations in patients with acute pancreatitis over the first three days of admission.

A) Serum soluble E-selectin concentrations in patients with mild (n=9) and severe (n=9) disease. The observed pattern of increasing serum soluble E-selectin concentration in patients with severe acute pancreatitis was significantly different from those with mild disease (ANOVA p=.044).

B) Serum soluble E-selectin concentrations with respect to mortality. The observed increase in serum soluble E-selectin concentrations in non-survivors (n=6) was significantly different from survivors (n=12) (ANOVA p=.001).

Values are mean with error bars indicating standard error of the mean (SEM).

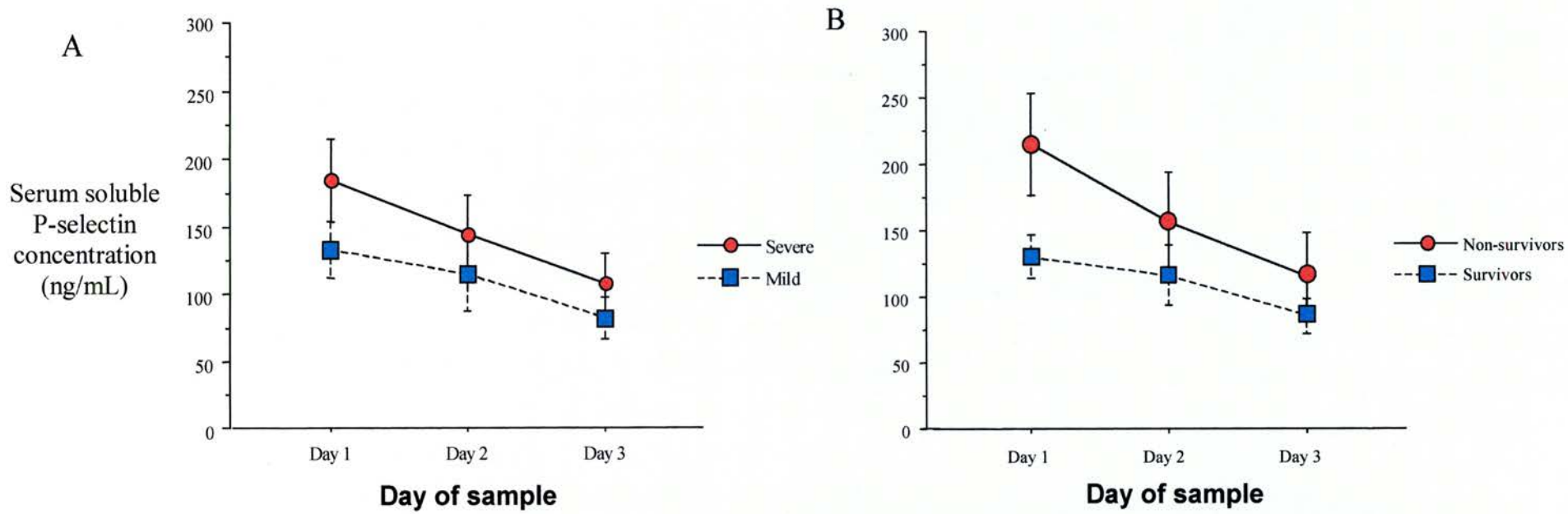


Figure 11: Serum soluble P-selectin concentrations in patients with acute pancreatitis over the first three days of admission.

A) Serum soluble P-selectin concentrations in patients with mild (n=9) and severe (n=9) disease. Over the three days the change in serum concentrations was significant (ANOVA $p<.001$) but not between patient groups.

B) Serum soluble P-selectin concentrations with respect to mortality. Over the first three days of admission, serum soluble P-selectin concentrations were significantly higher in non-survivors (n=6) than in survivors (n=12) (ANOVA $p=.005$). Values are mean with error bars indicating SEM.

Haematocrit And Platelet Counts

In patients with mild or severe pancreatitis there was a significant fall (ANOVA $p<.001$) in haematocrit over the three day study period (Figure 12A). The fall in haematocrit in patients with severe disease was significantly greater than the fall observed in patients with mild disease (ANOVA $p=.032$). There was a significant fall in platelet count in patients with mild and severe disease over the three day study period (Figure 12B. ANOVA $p<.001$). Patients with severe acute pancreatitis had lower platelet counts (ANOVA $p=.031$) than those with mild disease.

Stepwise backward logistic multiple factor regression analysis revealed no association between platelet count and serum soluble P-selectin levels (haematocrit $F=21.89$, white cell count $F=0.82$, platelet count $F=3.34$, intercept $F=5.07$; $p<.001$). There was a significant negative correlation between the platelet count and serum soluble E-selectin levels (haematocrit $F=0.12$, white cell count $F=0.13$, platelet count $F=7.98$, intercept $F=50.21$; $p=.007$).

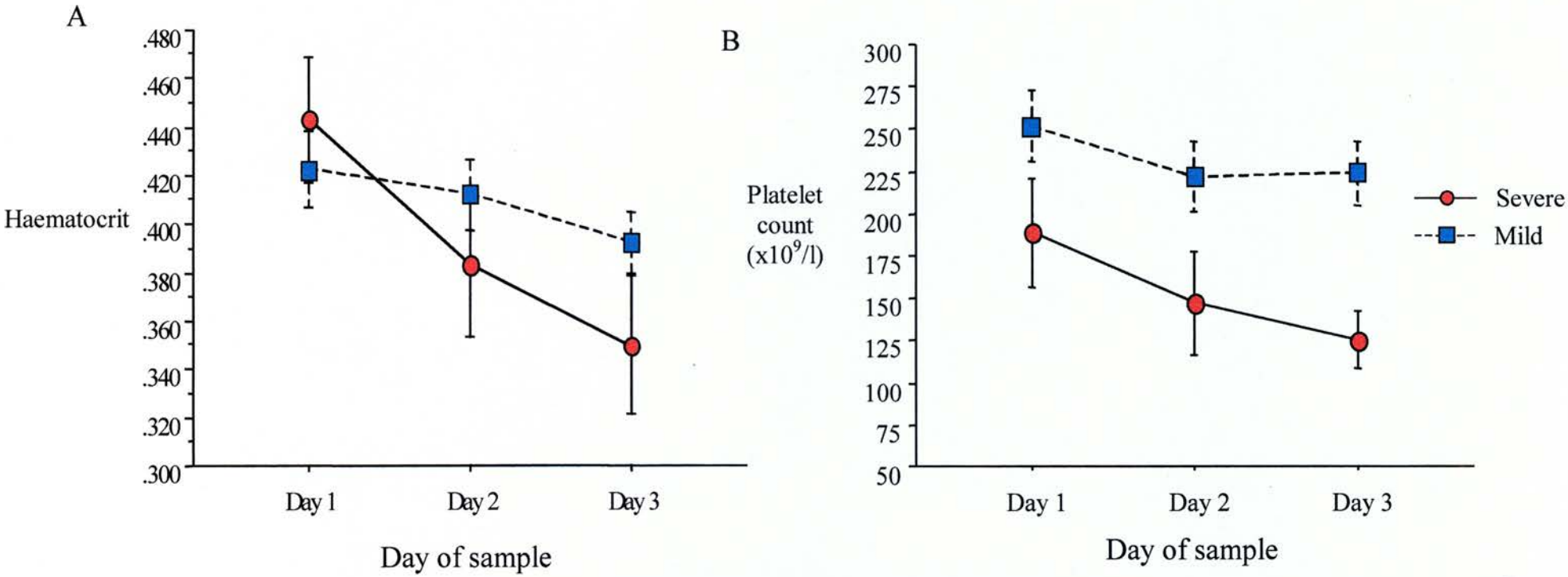


Figure 12. Haematological indices in patients with mild and severe acute pancreatitis

A) Haematocrit in patients with mild (n=9) and severe acute pancreatitis (n=9). The observed fall in haematocrit over the 3 days is significant (ANOVA $p<.001$). Further, the increased rate of decline in patients with severe disease when compared to the decline in those with mild disease is significant (ANOVA $p=.032$).

B) Platelet count in patients with mild (n=9) or severe (n=9) acute pancreatitis. The observed difference in platelet count between the two patient groups was significant (ANOVA $p=.031$). The change in platelet counts over time was also significant (ANOVA $p<.001$).
Values are mean with error bars indicating SEM.

Cytokine Gene Polymorphisms In Acute Pancreatitis

The frequency distribution of genotypes for the tumour necrosis factor gene polymorphisms studied is shown in Table 19. There was no significant difference in the distribution of tumour necrosis factor gene polymorphisms between patients with mild or severe disease. Similarly, no significant difference in the rate of carriage of the high secreting alleles was seen between the two patient populations. For the TNF-308 polymorphism, allele 2 occurred in 42/113 (0.37) of patients with mild disease compared with 26/77 (0.34) patients with severe disease (χ^2=0.23, p=.631; OR 95%C.I. 0.47-1.58). Likewise TNFB allele 2 was found in 97/113 (0.86) patients with mild disease compared with 68/77 (0.88) with severe disease (χ^2=0.25, p=.621; OR 95%C.I. 0.53-2.95). Further, there were no significant differences in the TNF-308 or TNFB genotype frequency distributions between patients with acute pancreatitis and healthy controls. For all subjects studied, individuals who were homozygous for TNFB allele 2 were always homozygous for TNF-308 allele 1 (χ^2=119.00, p<.001).

The frequency distribution of genotypes for the IL1β gene polymorphism is shown in Table 20. There were no significant differences in genotype frequency distribution between patients with mild or severe acute pancreatitis. Similarly, there was no significant difference in the rate of carriage of the allele linked with high secretion of IL1β. Allele 2 was found in 43/113 (.38) of patients with mild disease compared with 29/77 (.38) of patients with severe acute pancreatitis (χ^2=0.003, p=.957; OR 95%C.I. 0.54-1.78). No significant difference in genotype frequencies was observed between the patients with acute pancreatitis and healthy controls.

The frequency distribution of genotypes for the IL1RN gene polymorphism is shown in Table 21. No significant differences between the two patients groups were observed. Allele 2 of the IL1RN polymorphism was found in 63/113 (.56) of patients with mild disease compared with 35/77 (.46) with severe disease (χ^2=1.94, p=.163; OR 95%C.I. 0.37-1.18). Again no significant difference was observed

between patients with acute pancreatitis and healthy controls with respect to IL1RN genotype.

Analysis of data derived from all individuals demonstrated a significant interaction between IL1β alleles and IL1RN alleles, such that carriers of IL1β allele 2 had reduced carriage of IL1RN allele 2. ILIRN allele 2 was found in 48/117 (.41) of individuals carrying IL1β allele 2 compared with 98/175 (.56) of individuals without IL1β allele 2 (χ^2=6.29, p=.012). However, the carriage of the high secreting IL1β allele 2 along with non-carriage of the high secreting IL1RN allele 2 was not associated with disease severity (χ^2=0.73, p=.392; OR 95%C.I. 0.67-2.72)

Finally there was no significant association between aetiological agent and cytokine genotype for TNF-308 (χ^2=1.64, p=.950), TNFB (χ^2=2.50, p=.869), IL1B (χ^2=7.23, p=.300) and IL1RN (χ^2=26.40, p=.192). Likewise there was no significant association between the aetiological agent and the allele associated with high secretion for TNF-308 (χ^2=1.02, p=.797), TNFB (χ^2=1.21, p=.750), IL1B (χ^2=3.94, p=.267) and IL1RN (χ^2=0.98, p=.806).

Disease Severity [1]	TNF-308 genotype			TNFB genotype		
	Homozygous 1/1	Heterozygous 1/2	Homozygous 2/2	Homozygous 1/1	Heterozygous 1/2	Homozygous 2/2
Mild disease	71 (.62)	40 (.36)	2 (.02)	16 (.14)	48 (.43)	49 (.43)
Severe disease	51 (.66)	20 (.26)	6 (.08)	9 (.12)	41 (.53)	27 (.35)
		χ^2=5.32, p=.070			χ^2=2.14, p=.344	
Acute pancreatitis	122 (.64)	60 (.32)	8 (.04)	25 (.13)	89 (.47)	76 (.40)
Healthy controls	65 (.64)	32 (.31)	5 (.05)	16 (.16)	47 (.46)	39 (.38)
		χ^2=0.08, p=.964			χ^2=0.36, p=.834	

Table 19: Frequency distribution of the tumour necrosis factor gene polymorphisms.

Frequency distribution of the tumour necrosis factor gene polymorphism in patients with acute pancreatitis and healthy controls. Figures are the number of individuals with that genotype. The relative frequency of the genotype for mild or severe disease is in parentheses. Comparison between groups was carried out using the χ^2 test.

Disease Severity [1]	IL1β genotype		
	Homozygous 1/1	Heterozygous 1/2	Homozygous 2/2
Mild disease	70 (.62)	38 (.34)	5 (.04)
Severe disease	48 (.62)	28 (.37)	1 (.01)
		χ^2=1.52, p=.468	
Acute pancreatitis	118 (.62)	66 (.35)	6 (.03)
Healthy controls	57 (.56)	40 (.39)	5 (.05)
		χ^2=1.33, p=.514	

Table 20: Frequency distribution of the IL1β gene polymorphism.

Frequency distribution of the IL1β gene polymorphism in patients with acute pancreatitis and healthy controls. Figures are the number of individuals with that genotype. The relative frequency of the genotype for mild or severe disease is in parentheses. Comparison between groups was carried out using the χ^2 test.

Disease Severity [1]	IL1RN genotype								
	1/1	1/2	1/3	1/4	1/5	2/2	2/3	2/5	4/4
Mild disease	43 (.38)	52 (.46)	6 (.05)	0 (.00)	1 (.01)	9 (.08)	1 (.01)	1 (.01)	0 (.00)
Severe disease	38 (.49)	27 (.35)	3 (.04)	1 (.01)	0 (.00)	7 (.09)	1 (.01)	0 (.01)	0 (.00)
					χ^2=5.86, p=.556				
Acute pancreatitis	81 (.42)	79 (.41)	9 (.05)	1 (.01)	1 (.01)	16 (.08)	2 (.01)	1 (.01)	0 (.00)
Healthy controls	50 (.49)	33 (.33)	3 (.03)	0 (.00)	0 (.00)	13 (.13)	2 (.01)	0 (.00)	1 (.01)
					χ^2=7.72, p=.461				

Table 21: Frequency distribution of the IL1RN gene polymorphism.

Frequency distribution of the IL1RN gene polymorphism in patients with acute pancreatitis and healthy controls. Figures are the number of individuals with that genotype. The relative frequency of the genotype for mild or severe disease is in parentheses. Comparison between groups was carried out using the χ^2 test.

Cytokine Phenotype In Acute Pancreatitis

In non-stimulated whole-blood culture the production of TNFα and IL1β was below the lower limit of detection (Figure 13 and Figure 14). In contrast after 8 hours incubation with 5μg/mL of LPS, TNFα secretion was significantly increased in both patient groups (Wilcoxon signed rank p<.001). There was no significant difference in TNFα secretion between patients with previously mild [4136pg/mL (IQR 2996-4593)] and severe disease [3319pg/mL (IQR 3319-4833), Mann-Whitney U p=.932]. Similarly after 8 hours incubation there was a significant increase in IL1β secretion (Wilcoxon signed rank p<.001). Again the increase in IL1β secretion was similar between the two patient groups [mild 2612pg/mL (IQR 2223-4076) vs severe 2937pg/mL (IQR 1914-3400), Mann-Whitney U p=.604].

IL1RA secretion was significantly increased following LPS stimulation [unstimulated 285pg/mL (IQR 285-295) vs stimulated 2473pg/mL (IQR 1676-3230), Wilcoxon signed rank p<.001] (Figure 15). Although stimulated IL1RA secretion was higher in patients with severe disease the increase was not significant [mild 1995pg/mL (IQR 1289-3154) vs severe 2630pg/mL (IQR 2217-3394), Mann-Whitney U p=.057]. However, the ratio of IL1β to IL1RA secretion was significantly different between the two groups (Figure 16). Patients with previous mild acute pancreatitis had a higher IL1β:IL1RA ratio when compared to those with previous severe disease [mild 1.28 (IQR 1.01-2.29) vs severe 0.95 (IQR 0.73-1.79), Mann-Whitney U p=.016]. There was no correlation between IL1β and IL1RA secretion (r^2=.021 p=.306) (Figure 17).

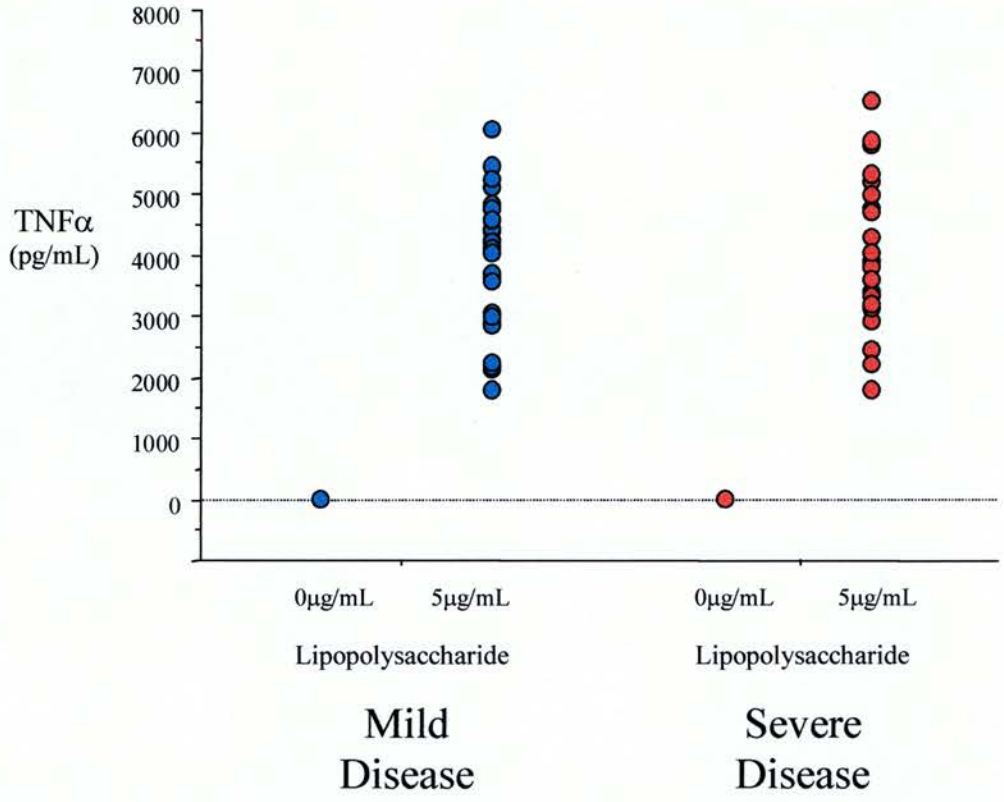


Figure 13: Plot of supernatant TNFα concentration in whole blood culture following recovery from acute pancreatitis.

Paired samples were either unstimulated or stimulated with 5μg/mL lipopolysaccharide. Each point represents a single individual.

In patients with previous mild (n=26) and severe (n=25) acute pancreatitis there was a significant increase in TNFα secretion following stimulation (Mild Wilcoxon signed rank p<.001, Severe Wilcoxon signed rank p<.001). There was no significant difference in stimulated TNFα secretion in patients with mild and severe disease (Mann-Whitney U p=.932).

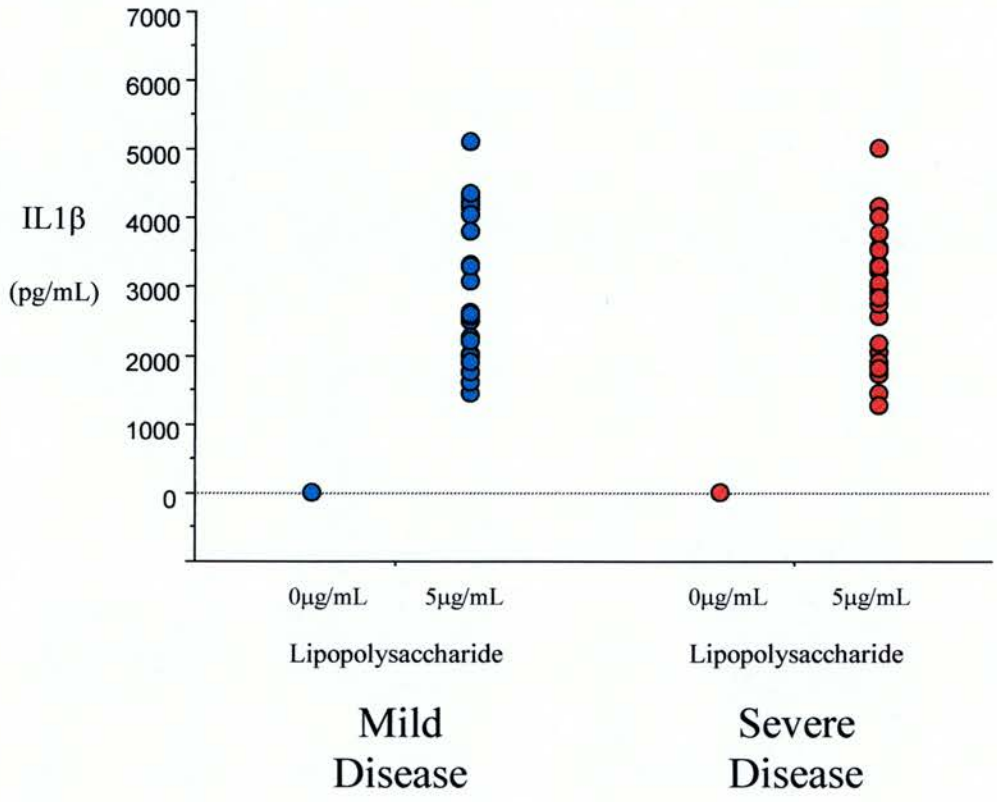


Figure 14: Plot of supernatant IL1β concentration in whole blood culture following recovery from acute pancreatitis.

Paired samples were either unstimulated or stimulated with 5μg/mL lipopolysaccharide. Each point represents a single individual.

In patients with previous mild (n=26) and severe (n=25) acute pancreatitis there was a significant increase in IL1β secretion following stimulation (Mild Wilcoxon signed rank p<.001, Severe Wilcoxon signed rank p<.001). There was no significant difference in stimulated IL1β secretion in patients with mild and severe disease.(Mann-Whitney U p=.604).

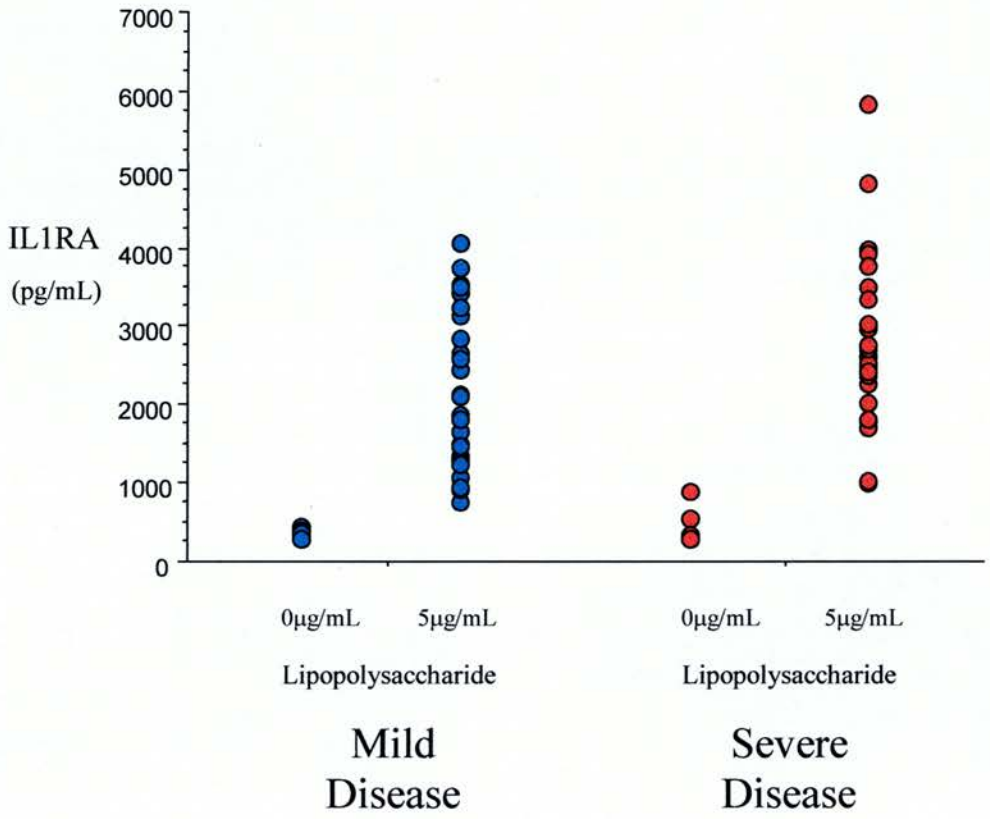


Figure 15: Plot of supernatant IL1RA concentration in whole blood culture following recovery from acute pancreatitis.

Paired samples were either unstimulated or stimulated with 5µg/mL lipopolysaccharide. Each point represents a single individual.

In patients with previous mild (n=26) and severe (n=25) acute pancreatitis there was a significant increase in IL1RA secretion following stimulation (Mild Wilcoxon signed rank p<.001, Severe Wilcoxon signed rank p<.001). Although stimulated IL1RA production was higher in patients with previous severe disease the difference was not significant (Mann-Whitney U p=.057).

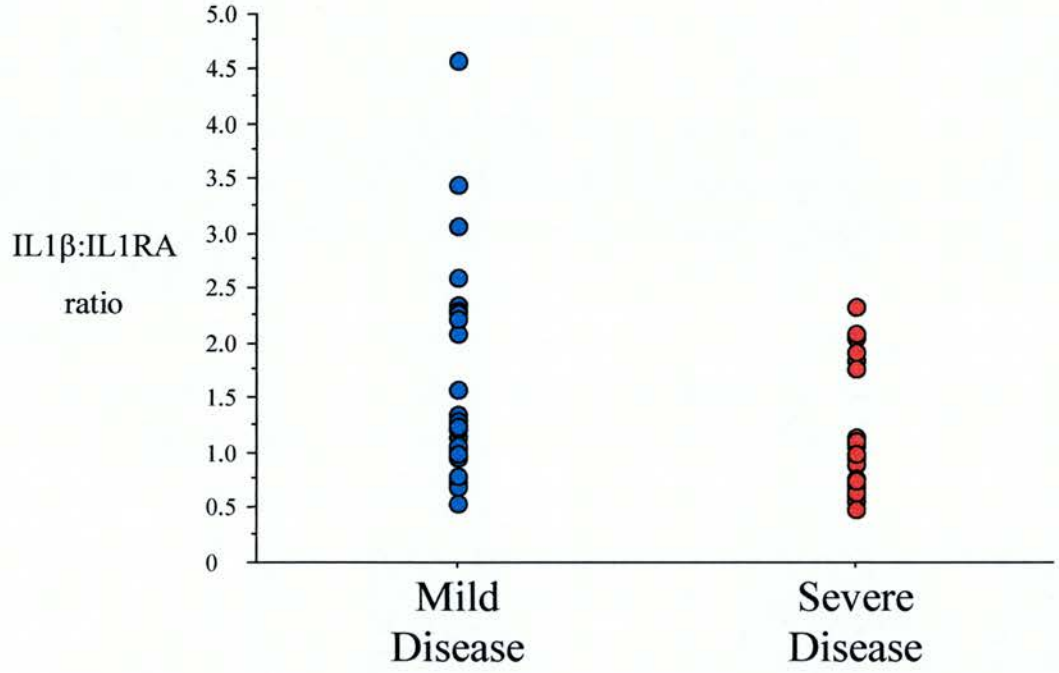


Figure 16: Plot of supernatant IL1β:IL1RA concentration ratio in whole blood culture following stimulation with 5μg/mL lipopolysaccharide.

Patients with previous mild acute pancreatitis (n=26) had a higher IL1β:IL1RA ratio when compared to those with previous severe disease (n=25) [mild 1.28 (IQR 1.01-2.29) vs severe 0.95 (IQR 0.73-1.79), Mann-Whitney U p=.016]

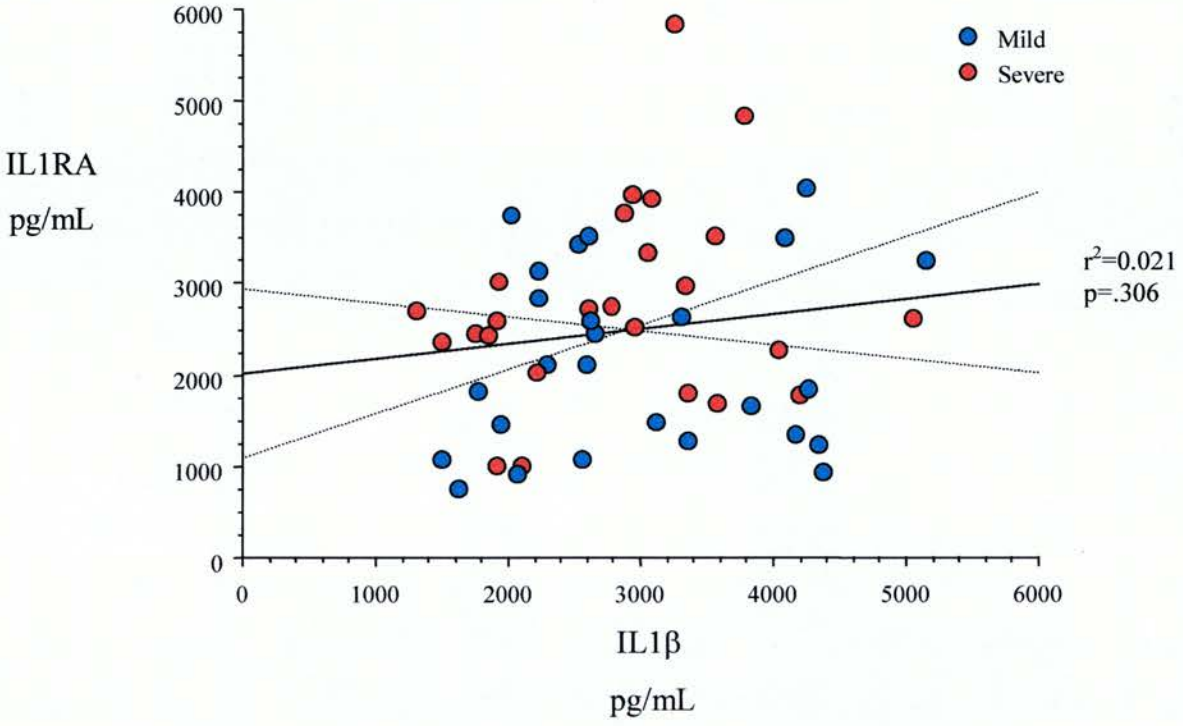


Figure 17: Bivariate plot of IL1β and IL1RA supernatant concentration.

Bivariate plot of IL1β and IL1RA production in whole blood cultures following incubation with 5μg/mL LPS. Individual points represent those with previous mild (●) or severe disease (●). Solid line represents the regression slope whilst dotted lines represent the 95% confidence intervals.

Haematological Indices And Cytokine Secretion

The differential full blood counts for the two patient groups are shown in Table 22. Comparison between the two patient groups revealed a significant difference in monocyte count between patient groups [mild $0.195x10^9$/L (IQR 0.150-0.280) vs severe $0.290x10^9$/L (IQR 0.210-0.333), Mann-Whitney U p=.030]. The normal reference range for monocyte count is 0.2 to $0.8x10^9$/L. There was no significant differences in any of the other haematological parameters between patients with mild and severe disease.

	Mild	**Severe**	
	n=26	n=25	
Neutrophils ($x10^9$/L)	4.25 (3.23-5.29)	3.87 (3.12-5.47)	Mann-Whitney U p=.699
Lymphocytes ($x10^9$/L)	1.71 (1.49-2.47)	2.07 (1.73-2.20)	Mann-Whitney U p=.155
Monocytes ($x10^9$/L)	0.20 (0.15-0.28)	0.29 (0.21-0.33)	Mann-Whitney U p=.030
Eosinophils ($x10^9$/L)	0.15 (0.11-0.24)	0.18 (0.12-0.27)	Mann-Whitney U p=.492
Basophils ($x10^9$/L)	0.04 (0.03-0.07)	0.06 (0.04-0.08)	Mann-Whitney U p=.318
Platelets ($x10^9$/L)	246 (219-262)	217 (190-291)	Mann-Whitney U p=.391
Haemoglobin (g/L)	137 (124-148)	137 (130-146)	Mann-Whitney U p=.706

Table 22: Differential full blood counts of patients participating in cytokine phenotype studies.

Stated values are median (interquartile range).

In order to identify potential relationships between haematological indices and cytokine concentrations in stimulated whole blood cultures, backward stepwise regression models were created. Within a stepwise regression model stimulated TNFα secretion was significantly associated with monocyte count

(F=4.50, p=.037; intercept F=79.31), but not associated with neutrophils (F=.03), lymphocytes (F=.36), eosinophils (F=.11), basophils (F=.02), platelets (F=.37) and haemoglobin (F=1.58). Therefore to correct for any influence of monocyte count on observed phenotype, corrected TNFα secretion was calculated. However, the production of TNFα per monocyte was similar between the two groups [mild 18517ng/mL/10^{10}monocytes/L (IQR 12226-29993) vs severe 12979ng/mL/10^{10}monocytes/L (IQR 10479-20046), Mann-Whitney U p=.077].

In contrast, stimulated IL1β production did not correlate with any haematological parameter [neutrophils F=.58, lymphocytes F=1.48, monocytes F=.08, eosinophils F=.46, basophils F=.25, platelets F=.08 and haemoglobin F=.51: intercept F= 26.00 p=.229].

IL1RA secretion correlated significantly with neutrophil count (F=27.75, p<.001; intercept F=4.36). ILIRA secretion did not correlate with any other parameter (lymphocyte F=.36, monocytes F=.57, eosinophils F=.71, basophils F=2.78, platelets F=.02 and haemoglobin F=.09). Therefore IL1RA concentrations were corrected for neutrophil count. With this correction IL1RA secretion was significantly higher in patients with previous severe disease when compared to those with previous mild disease [mild 470pg/mL/10^{10}neutrophils/L (IQR 352-652) vs severe 675pg/mL/10^{10}neutrophils/L (IQR 498-812), Mann-Whitney U p=.007].

Association Between Cytokine Phenotype And Genotype

No significant correlation was observed between TNFα secretion and TNF-308 genotype (Kruskal-Wallis p=.611), the carriage of TNF-308 allele 2 (Mann-Whitney U p=.784), TNFB genotype (Kruskal-Wallis p=.986) or the carriage of TNFB allele 2 (Mann-Whitney U p=.324).

Only two individuals were homozygous for allele 2 of the IL1β polymorphism, preventing analysis of the effect of IL1β genotype on IL1β secretion, however

carriage of allele 2 was not associated with increased secretion of IL1β (Mann-Whitney U p=.705).

Four IL1RN genotypes were observed, and no significant association between genotype and IL1RA secretion was detected (Kruskal-Wallis p=.582). Further, there was no association between the carriage of IL1RA allele 2 and IL1RA secretion (Mann-Whitney U p=.924).

Delivery Of Enteral Nutrition

Of fourteen patients randomised to receive enteral nutrition, nasojejunal tube placement was successful in 12 (86%). One patient randomised to receive enteral nutrition refused tube placement but was willing to participate in the study and therefore his results are included in the conventional therapy group. In the other patient the nasojejunal tube could not initially be passed through the pylorus but later passed spontaneously into the duodenum allowing enteral feeding. The duration of radiological screening was a median of 3.0 minutes (range 0.9-11.9). The median number of tube dislodgements was 1 (range 0-3) per person. Tubes remained in place for a median of 2 (range 1-7) days. The median number of tube placements was 1 (range 1-3) per person. The percentage of nutritional requirements delivered through enteral nutrition is shown in Figure 18. The delivery of enteral nutrition in the cohort remained relatively constant averaging a median 21% (range 0-100%) of predicted daily calorific requirement over the first 5 days. Over the first 5 days a median of 1.8 (range 0-7.3) megajoules/day was delivered. After this period the majority returned to normal diet. Patients unable to establish normal diet at this stage were also less able to tolerate enteral nutrition because of established ileus and this is reflected in the decrease in the percentage of calorific requirement delivered enterally after day 5. By day 10 no patient in this study was receiving artificial enteral nutrition. In 3 patients the volume of feed was limited by ileus. Three patients in this study received parenteral nutrition within the study period. Parenteral nutrition was commenced on day 4 in one patient in the enteral nutrition group because of profound ileus and high nasogastric aspirates (calorific requirements delivered by the parenteral route are excluded in this patient). Two patients in the conventional group commenced parenteral nutrition on days 2 and 7 respectively at the discretion of the treating physician.

For each patient group the volume of intravenous fluid therapy, oral fluid intake and urine output over the first four days of the study period is shown in Figure 19. There were no significant differences in intravenous fluid therapy (ANOVA p=.338), oral fluid intake (ANOVA p=.548) or urine volume (ANOVA p=.520) between the two patient groups. Further, as shown in Figure 20, urinary nitrogen excretion was similar between the two patient groups (ANOVA p=.497).

Normal diet was re-introduced at a median of 5 (range 4-9) days in patients receiving enteral nutrition compared with 6 days (range 4-10) for the conventional group (Mann-Whitney U p=.664). Two patients in the enteral group did not start normal diet by the end of the study period compared with 1 patient in the conventional group.

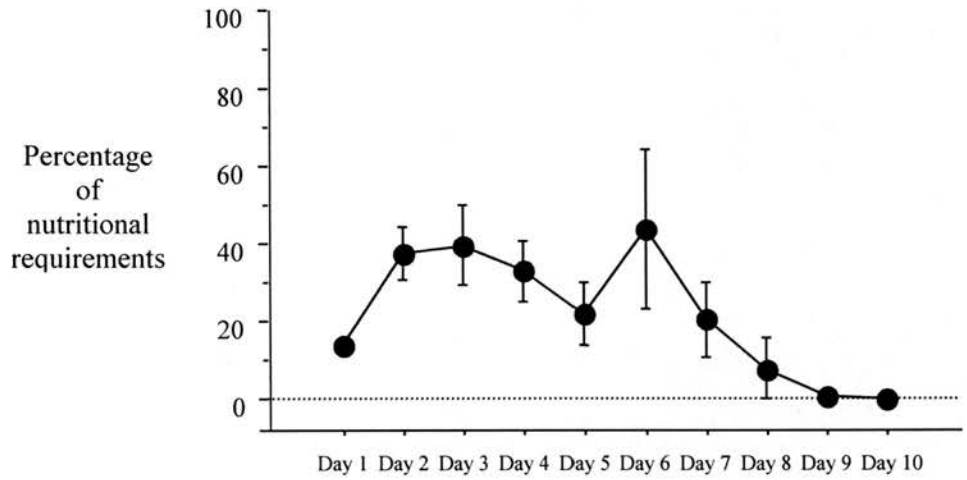


Figure 18: Percentage of nutritional intake as predicted by the Schofield equation delivered by nasojejunal feeding (n=13).
Points represent mean with error bars demonstrating standard error of the mean.

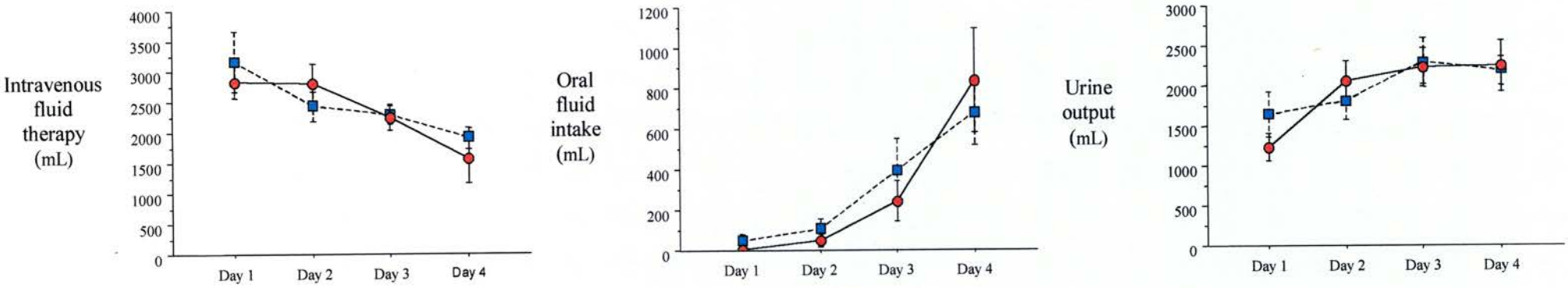


Figure 19: Volume of intravenous fluid therapy, oral fluid intake and urine output in patients receiving early enteral nutrition (n=13) (—o—) or conventional therapy (n=14) (--■--).

Values are mean with error bars representing standard error of the mean.

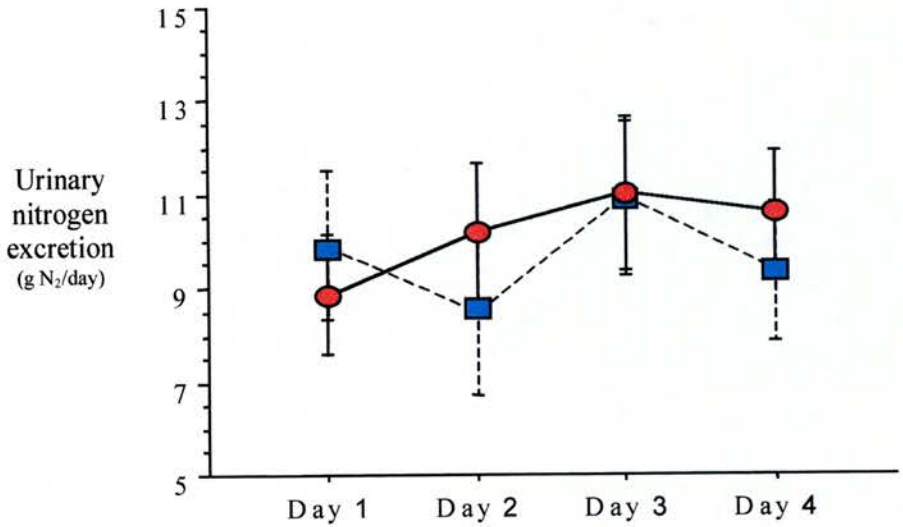


Figure 20: Urinary nitrogen excretion in patients receiving early enteral nutrition (n=13) (—●—) or conventional therapy (n=14) (--■--).

Values are mean with error bars representing standard error of the mean.

Change In Markers Of The Inflammatory Response

As the median delay to introduction of normal diet was 5 days and the aim of the study was to examine the effect of enteral nutrition on the systemic inflammatory response, statistical analysis was carried out on results obtained from the time of admission until day 4. Change in serum markers of the inflammatory response is seen in Figure 21. Serum IL6 levels fell significantly (ANOVA p=.009) during the study period in patients receiving enteral nutrition and in those receiving conventional treatment. Serum sTNFR-I remained relatively constant (ANOVA p=.952). Serum CRP concentrations increased from admission to day 2 and then fell (ANOVA p=.006). The introduction of enteral nutrition did not affect the concentrations of serum IL6 (ANOVA p=.281), serum sTNFR-I (ANOVA p=.531) or serum CRP (ANOVA p=.624) over the first 4 days of the study period.

Change in IgG anti-endotoxin core antibody antibody concentrations is shown in Table 23. The introduction of enteral nutrition did not significantly affect concentrations of IgG anti-endotoxin core antibody (Mann-Whitney U p=.846).

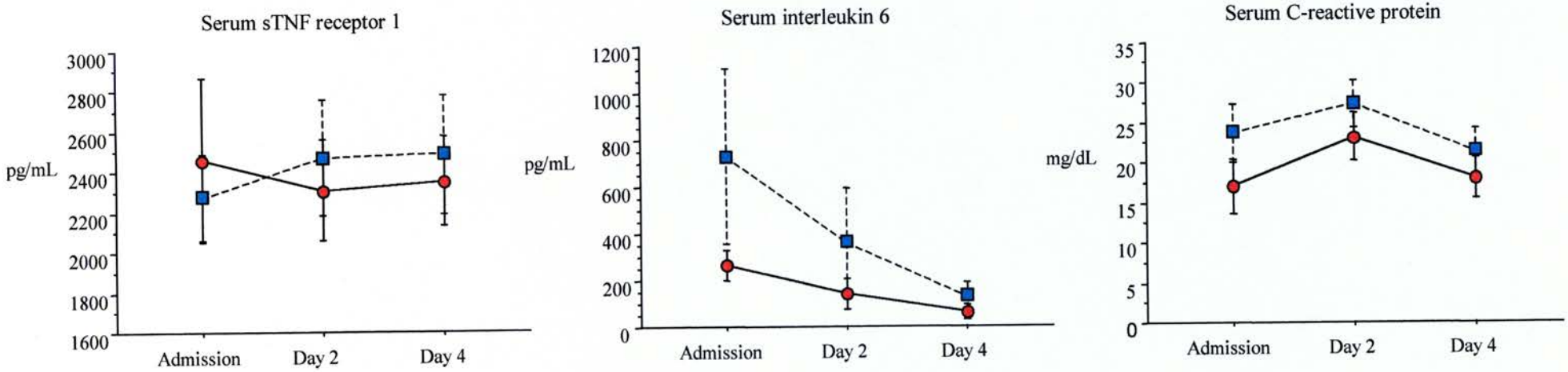


Figure 21: Concentration of serum sTNF receptor 1, interleukin 6 and C-reactive protein in patients receiving early enteral nutrition (n=13) (—●—) or conventional therapy (n=14) (--■--).

Values are mean with error bars representing standard error of the mean.

	Enteral	Conventional	
	Endocab IgG (MU/mL)	Endocab IgG (MU/mL)	
Admission	138 (60-554)	119 (29-205)	Mann-Whitney U p=.207
Day 4	142 (73-748)	103 (36-281)	Mann-Whitney U p=.081
Change of IgG antibody at day 4 as percentage of admission	6% (-61 to 567)	4% (-42 to 66)	Mann-Whitney U p=.846

Table 23: Concentration of anti-endocab IgG antibody during the first 4 days of admission.

Stated values are median (range).

Safety And Tolerance Of Early Enteral Nutrition

The results of nausea scores are shown in Figure 22. Although patients allocated to the enteral nutrition arm had higher nausea scores than patients in the conventional group (ANOVA p=.032), enteral nutrition did not significantly affect nausea (ANOVA p=.228).

One patient had probable feed-induced diarrhoea. There were no major complications that were directly attributable to enteral nutrition.

Gut Permeability To Non-Metabolised Sugars

The effect of enteral nutrition on gut permeability to non-absorbable sugars is shown in Table 24. In the group receiving enteral nutrition 4 patients had normal intestinal permeability at admission, however by the fourth study day 3 of these individuals had developed abnormal results (in the remaining individual sugar permeability studies were not possible). In contrast, 5 patients in the conventional group had abnormal results on admission and normal results on day 4.

Organ Dysfunction Scores

The Marshall organ dysfunction scores over the first four days of admission are shown in Table 25. Enteral nutrition did not affect organ dysfunction scores (ANOVA p=.521).

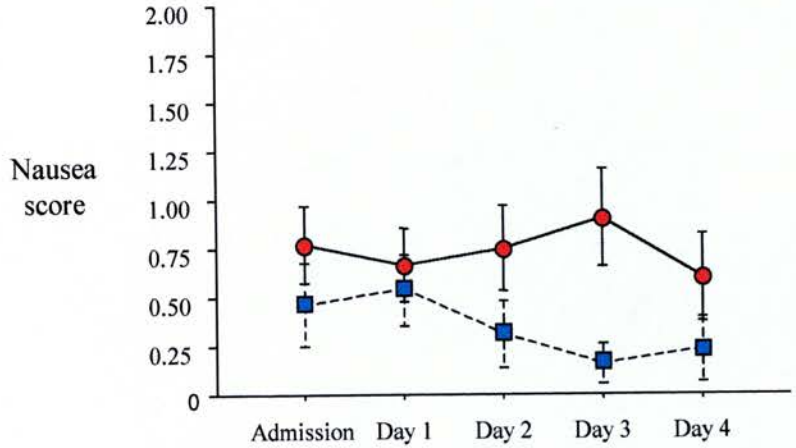


Figure 22: Nausea scores in patients receiving early enteral nutrition (n=13) (—●—) or conventional therapy (n=14) (--■--).

Values are mean with error bars representing standard error of the mean.

		Admission		Day 4	
		Enteral	Conventional	Enteral	Conventional
Intestinal	Normal	4	1	0	5
permeability	Abnormal	7	10	8	6
		χ^2=2.33, p=.127		χ^2=4.94, p=.026	

Table 24: Intestinal permeability as determined by differential sugar absorption at admission and on day 4.

	Marshall Multiple Organ Dysfunction Score [336]				
	Admission	Day 1	Day 2	Day 3	Day 4
Enteral nutrition	0 (0-4)	1 (0-3)	0 (0-6)	0 (0-6)	0 (0-7)
Conventional therapy	1 (0-8)	1 (0-6)	0.5 (0-11)	0 (0-11)	0 (0-10)

Table 25: Marshall organ dysfunction scores in patients receiving enteral nutrition and conventional therapy.

Stated values are median (range).

Discussion

Kinetics Of Serum Soluble E-Selectin And P-Selectin In Acute Pancreatitis

The present study demonstrates that during the first 3 days of admission, concentrations of serum soluble E-selectin increase in patients with severe acute pancreatitis, whilst remaining relatively constant in patients with mild disease. In contrast concentrations of serum soluble P-selectin fall significantly over the first three days, with no significant difference between patients with mild or severe acute pancreatitis, although levels were always higher in those with severe disease. However, serum P-selectin concentrations were significantly higher in non-survivors than survivors.

Although modern classification systems divide disease severity into mild or severe depending on a number of clinical criteria [1], in practice there is a continuum of disease severity. In order to observe the role of endothelial-derived selectins in acute pancreatitis the study sample consisted of patients at the extreme ends of the spectrum thereby minimising the sample size that would be required to detect any differences in serum soluble E-selectin and P-selectin concentrations. Although the degree of organ dysfunction has not been quantified, it is clear from the outcome of patients with severe acute pancreatitis that this patient group had profound organ dysfunction. Finally, by excluding patients with other active disease processes, it is likely that any differences observed between the two patient groups were due solely to the acute pancreatitis.

Because E-selectin is shed from endothelial cells following activation, soluble E-selectin has been used as a marker of endothelial cell [209]. Intravenous administration of tumour necrosis factor α [340] or lipopolysaccharide [341], both of which are implicated in the pathogenesis of severe acute pancreatitis [92, 96, 317-319, 342], cause dose-related increases in the levels of serum soluble E-selectin. Further, in critically ill patients with organ dysfunction arising from a systemic inflammatory response, levels of serum soluble E-selectin correlate with the degree of

haemodynamic compromise [343] and organ dysfunction [343-345]. Soluble E-selectin would therefore appear to be a reliable marker of endothelial cell activation in conditions such as acute pancreatitis in which organ dysfunction is a consequence of the systemic inflammatory response.

Previous reports regarding serum soluble E-selectin in acute pancreatitis have been incomplete. In a randomised placebo controlled trial of lexipafant [163], a platelet activating factor (PAF) antagonist, initial concentrations of serum soluble E-selectin were similar in patients with mild or severe disease; in keeping with the present observations. However, although levels of serum soluble E-selectin decreased over the following 2 days in patients receiving lexipafant or placebo, no comparison between patients with mild or severe disease was reported. In a similar fashion, Inagaki and colleagues [346] have reported that serum soluble E-selectin is increased 72 hours after pain onset, returning to normal at 168 hours. However, no comparison to patients with mild disease was made. In contrast to the previous two studies, in the present study significant differences between levels of serum soluble E-selectin between mild and severe acute pancreatitis were observed. These observations are in concordance with those of Hynninen and colleagues [347] who observed levels of serum soluble E-selectin in 9 patients with prognostically severe acute pancreatitis as defined by either an APACHE II score [329] of 9 or more, or a Balthazar grade [322] of D or E. Although mean levels of serum soluble E-selectin remained relatively constant over the first few days for the complete cohort, when analysed with reference to organ dysfunction severity scoring, patients with marked organ dysfunction developed significantly higher levels of serum soluble E-selectin on days 2 and 3, a pattern similar to that observed in the present study. Moreover the present observation that increased endothelial cell activation occurs in patients with severe acute pancreatitis when compared to those with mild disease is further corroborated by Chaloner and colleagues [348] who reported that levels of von Willebrand factor, another putative marker of endothelial cell activation, were higher in patients with severe acute pancreatitis when compared to patients with mild disease.

In contrast to the consensus belief that E-selectin is a specific marker of endothelial cell activation, the significance of serum soluble P-selectin levels is still controversial. Although P-selectin is found in the membrane of endothelial cell Weible-Palade bodies, it is also a constituent of the platelet α-granule membrane, demonstrating surface expression following platelet activation [349, 350]. Although soluble forms of P-selectin may arise either through proteolytic cleavage of cell surface P-selectin or through the production of alternatively spliced isoforms missing the transmembrane region [351], neither form appears to be exclusively produced by one cell type. Attempts at correlating levels of serum soluble P-selectin with other markers of either endothelial cell or platelet activation have also proved unhelpful in determining the source of serum soluble P-selectin in vivo [208, 352], possibly because P-selectin is a membrane constituent whilst the other markers measured are soluble components of secretory granules. Furthermore, in patients with the systemic inflammatory response syndrome it is likely that endothelial and platelet activation co-exist, therefore serum soluble P-selectin may be derived from either cellular source. The previously reported observations that signs of activation of the coagulation system, including reductions in platelet counts, are common in patients with acute pancreatitis suggests that concomitant endothelial cell and platelet activation occurs in acute pancreatitis [353-355].

Although it is not possible to confirm the exact source of serum soluble P-selectin in patients with acute pancreatitis, the observation that serum soluble P-selectin concentrations do not correlate with the platelet count suggest that it is derived mainly from endothelial cells. If the measured serum soluble P-selectin was derived from platelets it may be expected that P-selectin levels would be highest in the patients with the greatest levels of platelet activation. In the present patient population, those with the highest level of platelet activation would be those who paradoxically had the lowest platelet count as a result of widespread activation of the coagulation system [356]. In keeping with this supposition, platelet count demonstrated a significant negative correlation with concentrations of serum soluble E-selectin, suggesting platelet activation and consumption alongside endothelial activation. In contrast P-selectin concentration did not correlate with platelet count.

Although no significant difference in P-selectin levels was observed between patients with mild or severe disease, the observation that levels were significantly higher in non-survivors and that serum concentrations fell over the first three days suggest that P-selectin has a role in the pathogenesis of acute pancreatitis. Further supporting the role of P-selectin in the pathogenesis of severe acute pancreatitis is the observation that in an experimental model of acute pancreatitis up-regulation of P-selectin expression occurs in the lungs and that immunoneutralisation of P-selectin ameliorates pulmonary neutrophil infiltration [190].

Within the present study there appeared to be differential expression of endothelial-derived selectins. P-selectin expression occurred early in all patients with acute pancreatitis, with increased E-selectin expression occurring later and confined to those with severe disease. It is not clear what mechanisms are responsible for generalised expression of P-selectin but selective expression of E-selectin. However, the observed temporal patterns of serum soluble selectins would be in keeping with the previously observed early expression of pre-formed and stored P-selectin and the late expression of E-selectin following *de-novo* synthesis. Moreover the present observations of differential selectin expression are in keeping with those derived from an experimental model. Using a dual radio-labelled monoclonal antibody technique, Lundberg and colleagues [210] demonstrated early expression of P-selectin in all major organs bar the kidney, whilst expression of E-selectin occurred later and was confined to the lungs. Interestingly the expression of P-selectin appeared to be biphasic with a further late peak coinciding with the peak of E-selectin and presumed to be due to late *de-novo* synthesis of P-selectin. The failure to observe such a biphasic pattern of serum soluble P-selectin concentrations within the present study may be due to the relatively short duration of sampling.

In conclusion, the present study provides evidence of a strong association between endothelial-derived selectins and the development of organ dysfunction in acute pancreatitis. In addition the results confirm the importance of endothelial cell

activation as part of the systemic inflammatory response in patients with acute pancreatitis.

Cytokine Genotype And Phenotype In Acute Pancreatitis

Although environmental factors trigger cytokine secretion, genetic factors may be important in determining levels of secretion [225]. *In vitro* studies have identified that individuals may demonstrate consistent differences in leukocyte cytokine secretion and that these differences are probably genetically pre-determined [225]. Genetically-determined differences in levels of cytokine secretion following a stimulus may explain individual differences in disease severity in inflammatory disorders such as acute pancreatitis.

The present observational study tests the hypothesis that patients with severe acute pancreatitis have higher levels of pre-determined pro-inflammatory cytokine secretion than patients with mild disease by examining the distribution of cytokine gene polymorphisms associated with variation in TNFα, IL1β and IL1RA secretion. The results show no correlation between the gene polymorphisms studied and disease severity. Further, comparison of genotype frequencies in patients with acute pancreatitis and in healthy individuals suggests that these polymorphisms do not influence disease susceptibility. Finally, within the present study there was no correlation between the cytokine gene polymorphisms studied and levels of *in vitro* cytokine production.

Within the limits of current evidence it is not possible to state with certainty whether the polymorphisms studied are the most important markers of genetically determined cytokine secretion. Therefore in order to ascertain whether patients with severe acute pancreatitis are indeed "high secretors" of cytokines, phenotype studies were undertaken. These studies were carried out following recovery from an episode of acute pancreatitis thereby minimising the influence of environmental factors and leukocyte down-regulation during the acute illness [332, 357]. Clearly, non-survivors

were not included in this group and it is not possible to exclude different patterns of responsiveness in patients dying of acute pancreatitis. However, within these limitations the present results demonstrate that levels of leukocyte TNFα secretion are similar in patients with previously mild or severe acute pancreatitis. However, although IL1β and IL1RA secretion was similar in the two patient groups, a significant difference in the IL1β:IL1RA ratio was observed such that patients with previously mild disease had an increased ratio. This difference may be the result of increased secretion of IL1RA in patients with severe acute pancreatitis.

Monocyte secretion of TNFα is increased in patients with severe acute pancreatitis. McKay and colleagues [99] have demonstrated increased monocyte secretion of TNFα in patients with severe acute pancreatitis. The observation that following recovery leukocyte secretion of TNFα is similar in all patients suggests that environmental factors are the principal modulators of leukocyte TNFα secretory responses.

There are several potential reasons for failing to observe any association between cytokine gene polymorphisms and levels of cytokine secretion. Although polymorphisms may only be markers of other functionally significant gene polymorphisms at least one of the polymorphisms examined in this study is known to have functional significance [213-215, 217]. The TNF-308 gene polymorphism would appear to occur at a transcription factor binding site in the TNFα promoter [213, 214] and therefore affects protein binding thereby altering transcriptional activity [214]. However, other data including the present study have cast doubt upon the functional significance of the TNF-308 polymorphism [216, 224, 225], although differential TNFα secretion may be cell and stimulus specific [217]. Although the failure of the present study to observe differential TNFα secretion associated with the TNF-308 polymorphism may be due to an inappropriate stimulus other possibilities exist. In complex biological systems the effect of a single gene polymorphism in determining cytokine secretion may be minimised through the interaction of other factors. The increased potential for interacting systems in whole blood culture may explain the failure of the present study and others research groups to demonstrate an association between TNF gene polymorphisms and increased secretion of TNFα in whole blood

culture [224, 225]. It is perhaps, therefore, not surprising that single gene polymorphisms do not correlate with outcome in patients with severe acute pancreatitis.

One other study has investigated interleukin 1 gene polymorphisms in patients with acute pancreatitis [252]. As in the present study, no association was found between the IL1β *Taq1* polymorphism and disease susceptibility and severity. However, in contrast to the present study, Smithies observed an increased rate of carriage of IL1RN allele 1 in patients with acute pancreatitis when compared to healthy individuals, and an increased rate of carriage of IL1RN allele 1 in patients with severe acute pancreatitis when compared to those with mild disease [252]. These observations led to the suggestion that the IL1RN gene polymorphism has a role in determining both disease susceptibility and severity. However, if the present observations are combined with the results published by Smithies and colleagues, IL1RN genotype has no association with disease susceptibility (χ^2=9.44, p=.223) or severity (χ^2=7.14, p=.414), nor does the carriage of IL1RN allele 1 have an association with disease susceptibility (χ^2=3.67, p=.055) or severity (χ^2=0.31, p=.580). It would therefore appear that the IL1RN VNTR polymorphism does not play a major role in the pathogenic mechanisms of acute pancreatitis.

Although the observation of an increased ratio of IL1β to IL1RA in patients with mild acute pancreatitis would appear to be paradoxical, it may be a real and important biological observation. Heresbach and colleagues [358] have recently reported that during an episode of severe acute pancreatitis patients have a reduced serum IL1β:IL1RA ratio. This alteration in the IL1β:IL1RA ratio in patients was due mainly to an increase in secretion of IL1RA rather than a reduction in IL1β secretion. These observations are in keeping with the present results in which there was no difference in IL1β secretion between the two groups but a trend towards increased secretion of IL1RA in patients with previous severe disease leading to a significantly lower IL1β:IL1RA ratio. Further, when corrected for differences in differential blood counts, patients with previous severe disease had significantly greater secretion of IL1RA than those with mild disease. It would appear logical that a lower IL1β:IL1RA ratio would be associated with a lesser inflammatory response

than higher ratios and therefore the present observation and that of Heresbach would appear to be paradoxical given that organ dysfunction in acute pancreatitis is believed to be due to a systemic inflammatory response. However, the relationships between IL1β, IL1RA and both membrane associated and soluble interleukin 1 receptors in determining a biological response is as yet unclear. For example, although IL1RA is a pure antagonist on membrane associated interleukin 1 receptors, it can bind to soluble interleukin 1 receptors, displacing bound bioactive IL1β [359]. Further, the importance of anti-inflammatory mechanisms in determining outcome in inflammatory conditions may be underestimated and poorly understood. Evidence exists suggesting that an excessive anti-inflammatory response in septic patients is detrimental to outcome [69, 76, 360] and may be the result of innate differences [225]. Taken together with the findings of Heresbach [358], the present observations suggest that pre-determined individual differences in the interleukin 1 system may be involved in mediating disease severity in acute pancreatitis. However, because cytokine phenotype was not determined during the in-hospital stay, no association between cytokine phenotype during and after and episode of acute pancreatitis can be conclusively assumed to exist, and therefore further studies are required to confirm the significance of the present observations before a pre-determined association is definitively established.

Finally, this study has only examined the genotype and phenotype of TNFα, IL1β and IL1RA and it is likely that further functional gene polymorphisms will be described which may have a role in determining disease severity in acute pancreatitis.

In conclusion the present study demonstrates that polymorphisms at the TNF and IL1 gene loci do not have a principal role in the regulation of disease severity in acute pancreatitis. In complex biological disease processes such as acute pancreatitis it is unlikely that single gene polymorphisms will regulate disease outcome. Further, as the present study of leukocyte TNFα and IL1β secretory phenotype in patients following an episode of acute pancreatitis shows no difference in leukocyte responsiveness between patients with mild and severe disease, it is

unlikely that any future identification of functional polymorphisms at these gene loci will alter the role of single gene polymorphisms in regulating outcome in this disease. In contrast, the present findings of a reduced IL1β:IL1RA ratio in patients with severe disease suggests that future studies should focus on the complex interaction between pro-inflammatory and anti-inflammatory mechanisms regulating the inflammatory process in acute pancreatitis.

Enteral Nutrition In Prognostically Severe Acute Pancreatitis

A principal focus of current research interest in severe acute pancreatitis is the development of effective therapies based on an improved understanding of the pathogenic mechanisms of this disease. In view of experimental and clinical evidence suggesting that gut dysfunction may contribute to the systemic inflammatory response seen in patients with acute pancreatitis a rational approach is the development of therapies limiting the effects of fasting and critical illness on the intestinal tract. Because studies in other critical illness states have demonstrated a reduction in infective complications in patients receiving enteral nutrition it is rational to hypothesise that similar benefits may be obtained in patients with acute pancreatitis. However, valid concerns exist regarding the potential for adverse stimulation of the inflamed pancreas by the presence of nutrition in the upper gastrointestinal tract. Moreover the feasibility of enteral feeding in patients with severe acute pancreatitis, in whom intestinal ileus may be a significant problem, has not been fully determined. Therefore the present study has focused on the practicalities of instituting early enteral nutrition in patients with prognostically severe acute pancreatitis as well as the effects of enteral nutrition on surrogate markers of the inflammatory response.

In order to minimise any potential pancreatic stimulation feed was delivered via nasojejunal tubes positioned radiologically beyond the ligament of Treitz [361]. Initial tube placement was successful in 86% of patients comparing favourably with a large series of fluoroscopically-placed tubes reported by Gutierrez [362]. However, although

successful tube placement can be readily achieved the present data suggest that tube dislodgement is common, reducing tube lifespan. Indeed in the present study the median tube lifespan was considerably shorter than may be expected from other published data [363]. However, despite difficulties in maintaining tube position, no significant complications occurred that could be directly attributable to enteral feeding, suggesting that nutritional support using the enteral route is safe. One practical solution to the problem of naso-enteric tube dislodgement may be the use of endoscopically placed clips securing the feeding tube to the bowel wall (CW Imrie – personal communication). A further solution to the problem of nasojejunal feeding is the use of nasogastric feeding tubes, which although remain prone to dislodgement are simpler to site. A recent observational study has suggested that nasogastric feeding is feasible, with acceptable rates of failure due to gastric stasis [364].

In the present study no effect of early enteral nutrition on markers of the inflammatory response or on organ dysfunction in patients with predicted severe acute pancreatitis was observed. This suggests that enteral nutrition at the level provided in this study does not ameliorate, and probably more importantly does not exacerbate, the inflammatory response in acute pancreatitis. The present observations are however different from previously reported studies. Windsor and colleagues [326] have reported a reduction in the systemic inflammatory response and in surrogate markers of bacterial translocation in 34 patients with acute pancreatitis receiving enteral nutrition when compared to those receiving parenteral nutrition. Similarly McClave [325] has reported a more rapid normalisation of the physiological disturbance in 30 patients receiving enteral nutrition when compared to those receiving parenteral nutrition. The differences between the present study and the other two may arise for several reasons.

Firstly and perhaps most importantly a median of 1.8 megajoules/day was delivered which constituted a median of 21% of daily calorific requirement. Although, the original intention was to provide as close to complete calorific requirements as possible, a cautious step-up protocol was selected to avoid feed-induced nausea, vomiting and the risk of feed aspiration. As reflected by the lack of difference in

nausea scores between the two patient groups and the absence of major feed-induced complications this strategy appears to have been successful. However, Windsor delivered approximately 65% of non-protein calories over the study period [326], whilst McClave provided 71% of caloric requirements [325]. Although the high rate of tube dislodgement may account for some of the discrepancy between ourselves and the other two studies, the difference in disease severity of patients in the two studies when compared to the present patient population may also be a factor. The patient population in the studies reported by Windsor and McClave had lower serum C-reactive protein concentrations and multiple factor prognostic scores than patients included in the present study indicating that the present study consisted of a patient population with greater disease severity. The preponderance of patients with mild acute pancreatitis in the studies of Windsor and McClave suggest that the majority of patients had minimal intestinal ileus and therefore maximal rates of feeding could be achieved without difficulty. In the present population of patients with more severe disease intestinal ileus is a significant problem, indeed in almost 25% of patients feed volumes had to be limited because of intestinal ileus. Although Kalferentzos and colleagues have demonstrated that 74% of calorific requirements could be supplied through enteral nutrition in severe acute pancreatitis this value was the mean value over a period of 35 days [327]. No value for the initial days following admission is provided by Kalferentzos, and it is likely that intestinal ileus is maximal just after admission to hospital. Indeed with respect to early intestinal ileus it is interesting to note that although McClave provided 71% of nutritional requirements during the total study period only 30% could be provided during the first 4 study days.

However, it is not clear whether delivery of full caloric requirements by enteral nutrition is required to obtain benefit. It has been postulated that so-called minimal enteral nutrition is able to provide enough luminal nutrition to maintain intestinal function thereby limiting the effects mediated by the intestinal tract in the systemic inflammatory response syndrome. Experimental studies have demonstrated that minimal enteral nutrition maintains intestinal mass but does not necessarily affect intestinal tract immune function [365, 366]. However, the addition of minimal enteral nutrition to parenteral nutrition in neonates requiring surgery is associated with an

improvement in systemic immune function [367]. The present results however suggest that minimal enteral nutrition does not modify the inflammatory response. More interestingly the present study observed an alteration in intestinal function as measured by intestinal permeability in patients receiving enteral nutrition. This observation is in contrast to evidence suggesting that enteral nutrition improves intestinal permeability in critical illness states [275]. The mechanisms responsible for this apparent deterioration of intestinal permeability are not clear. Although hypertonic solutions can cause an increase in intestinal permeability [368, 369], Jevity is an isotonic solution. Bacterial colonisation secondary to the enteral feeding tubes is another possible explanation but the apparent increase in abnormal intestinal permeability in patients receiving early enteral nutrition was not paralleled by altered IgG endotoxin antibody levels suggesting that there was no concomitant increase in bacterial translocation.

Another major difference between the present study and those of Windsor and McClave is the use of parenteral nutrition [325, 326]. In line with published guidelines, patients in the present study did not receive parenteral nutrition from admission. In contrast, in both of the other studies patients in the parenteral nutrition group received nutritional support from admission. Parenteral nutrition was therefore given to patients with mild disease, a group of patients that do not require routine artificial nutritional supplementation. More significantly however, Windsor and colleagues maintained a nil-by-mouth regime in the group receiving parenteral nutrition until the seventh study day irrespective of the clinical condition, therefore a group of patients remained fasted despite clinical resolution of the disease. The delivery of parenteral nutrition to patients with mild disease and the abstinence until the seventh day may account for the observed differences. Fong and colleagues [370] have demonstrated that bowel rest and the institution of parenteral nutrition in normal volunteers is associated with an increased inflammatory response following a stimulus, whilst Welsh and colleagues [371] have reported that malnourished patients have an impairment of intestinal function and increased markers of the acute phase response. Therefore observed differences between patients receiving parenteral or enteral

nutrition may not arise from a modulation of the disease process but may in fact be a direct consequence of the treatment modality.

Another reason for the failure to observe any effect on the inflammatory response may be a result of the earlier sampling on day 4 in the present study in comparison to day 7 as in the trial reported by Windsor. Measurement of the inflammatory response at day 4 may reflect the level of pancreatic inflammation and may be marginally be influenced by the effects of bacterial translocation. Therefore by day 4 the effects of enteral nutrition may not yet be apparent.

Although McClave [325] reported an improvement in the physiological disturbance as manifested by normalisation of Ranson's criteria [372] no effect of enteral nutrition on organ dysfunction as measured by the Marshall score was observed in the present study. However, the median organ dysfunction score was 1 out of a possible 24 points indicating that in a mixed population of intensive care and high dependency unit patients the Marshall organ dysfunction score does not have sufficient sensitivity. Although serial Ranson's [372], Glasgow [321] and APACHE II [329] scores have been used to monitor physiological disturbance in acute pancreatitis none of these systems was developed or validated for this purpose. An accurate physiological scoring system for this population of patients is therefore required.

Although surrogate markers such as C-reactive protein and organ dysfunction scores are frequently used, benefits in these measure are not necessarily sufficient to mandate a change in management. Of more importance are outcome measures such as mortality and major complications. Therefore the results from Kalferentzos' group demonstrating a reduction in total and septic complications from the use of enteral nutrition in patients with severe acute pancreatitis are exciting [327]. However, the ascribed benefits need to be confirmed in a larger study.

In summary, the present study demonstrates that although enteral nutrition is feasible in patients with prognostically severe acute pancreatitis, early enteral nutrition does not ameliorate or exacerbate the inflammatory response. With a

growing body of experience attesting to the clinical safety of this technique, and other small trials suggesting clinical benefit, a large multicentre trial is required to evaluate this treatment modality before widespread introduction into everyday clinical practice.

Summary

The present studies have provided further insight into the pathogenic mechanisms in acute pancreatitis and have evaluated a novel therapeutic intervention.

Through the determination of the kinetics of serum soluble E and P-selectin, evidence has been provided confirming the role of the endothelium in the mediation of severe acute pancreatitis. Moreover a differential pattern of serum soluble endothelial-derived selectins has been observed which is in keeping with the known biology of endothelial-derived selectins.

Further, the basis for the differential pattern of cytokine secretion observed in patients with mild and severe acute pancreatitis has been examined. To this end the frequency of polymorphisms which are associated with levels of cytokine secretion have been determined. No association was observed between tumour necrosis factor and interleukin 1 gene polymorphisms and disease severity within the present study population. However, cytokine secretory phenotype studies performed following an episode of acute pancreatitis phenotype studies have suggested that a pre-determined difference exists in the interaction of the pro-inflammatory and anti-inflammatory systems in patients with mild or severe acute pancreatitis. Future research should be directed at understanding the importance of anti-inflammatory mechanisms and their interaction with systemic pro-inflammatory mechanisms in determining the outcome of critical illness states.

Finally, the results of a phase II/III randomised trial suggest that early enteral nutrition is feasible in prognostically severe acute pancreatitis. However, no significant outcome benefits were observed following the introduction of enteral nutrition, although the small numbers included in the study preclude any definitive conclusions. A large multicentre randomised trial is required before this therapeutic strategy becomes the standard of care.

Appendices

Definitions

Throughout this thesis the terminology used is concordant with the definitions agreed at the International Symposium on Acute Pancreatitis held in Atlanta, USA in September 1992 (Atlanta Consensus Conference) [1]. This conference aimed to standardise the terminology used in acute pancreatitis thereby providing a framework upon which future research could be carried out.

Acute Pancreatitis

Acute pancreatitis is an acute inflammatory process of the pancreas, with variable involvement of other regional tissues or remote organ systems.

Mild acute pancreatitis

Mild acute pancreatitis is associated with minimal organ dysfunction and an uneventful recovery, and it lacks the described features of severe acute pancreatitis.

Severe Acute Pancreatitis

Severe acute pancreatitis is associated with organ failure and/or local complications such as necrosis, abscess or pseudocyst. Organ failure is defined as shock (systolic blood pressure less than 90mmHg), pulmonary insufficiency (PaO_2 8kPa or less), renal failure (creatinine level greater than 177μmol/L after rehydration) or gastrointestinal bleeding (more than 500mL/24 hours). Systemic complications such as disseminated intravascular coagulation (platelets $100x10^{-3}$/mL and/or fibrinogen less than 1.0g/L and fibrin split products more than 80μg/mL) or severe metabolic disturbance (serum calcium 1.87mmol/L or less).

Acute Fluid Collections

Acute fluid collections occur early in the course of acute pancreatitis, are located in or near the pancreas and always lack a wall of granulation or fibrous tissue.

Pancreatic Necrosis

Pancreatic necrosis is a diffuse or focal area(s) of nonviable pancreatic parenchyma which is typically associated with peri-pancreatic fat necrosis. Contrast-enhanced computed tomography is the current gold standard for the clinical diagnosis of pancreatic necrosis.

Acute Pseudocyst

A pseudocyst is a collection of pancreatic juice enclosed by a wall of fibrous or granulation tissue which arises as a consequence of acute pancreatitis, pancreatic trauma or chronic pancreatitis.

Pancreatic Abscess

A pancreatic abscess is a circumscribed intra-abdominal collection of pus usually in proximity to the pancreas containing little or no pancreatic necrosis which arises as a consequence of acute pancreatitis or pancreatic trauma.

Abbreviations

95%CI	95% confidence interval
APACHE II	Acute physiology and chronic health evaluation II
CARS	Compensatory anti-inflammatory response syndrome
CRP	C-reactive protein
CT	Computed tomography
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunoabsorbant assay
ERCP	Endoscopic retrograde cholangio-pancreatography
HLA-DR	Human leukocyte antigen class DR
ICAM-1	Intercellular adhesion molecule-1
IL1	Interleukin 1
IL1α	Interleukin 1α
IL1β	Interleukin 1β
IL10	Interleukin 10
IL1B	Interleukin 1β polymorphism in exon 5
IL1RA	Interleukin 1 receptor antagonist
IL1R-I	Interleukin 1 receptor type 1
IL1R-II	Interleukin 1 receptor type 2
IL1RN	Interleukin 1 receptor antagonist gene
IL6	Interleukin 6
IL8	Interleukin 8
IQR	Interquartile range
ISD	Information and Statistics Division
L/R ratio	Lactulose/rhamnose ratio
LPS	Lipopolysaccharide
MAP kinase	Mitogen-activated protein kinase
MODS	Multiple organ dysfunction syndrome
mRNA	Messenger ribonucleic acid
NFκB	Nuclear factor κB
NSAIDs	Non-steroidal anti-inflammatory drugs
OR	Odds ratio
PAF	Platelet activating factor
PCR	Polymerase chain reaction
PEG	Polyethylene glycol

RFLP	Restriction fragment length polymorphism
RPMI	Roswell Park Memorial Institute
SEM	Standard error of the mean
SIRS	Systemic inflammatory response syndrome
SMR	Scottish Morbidity Record
sTNFR-I	Soluble tumour necrosis factor receptor type 1
sTNFR-II	Soluble tumour necrosis factor receptor type 2
TNF	Tumour necrosis factor
TNFα	Tumour necrosis factorα
TNFβ	Tumour necrosis factorβ
TNF-308	Tumour necrosis factor α polymorphism at position –308
TNFB	Tumour necrosis factor β polymorphism in intron 1
UK	United Kingdom
USA	United States of America
VNTR	Variable number tandem repeat

Suppliers of materials

Anti-endotoxin core antibody ELISA	Coaset Endocab Chromogenix, Mölndal Sweden
Cell culture plates	Corning Costar, High Wycombe UK
Corsafe® nasojejunal feeding tube	Corsafe Merck, Middlesex UK
C-reactive protein fluorometric immunoassay	Abbott, Berkshire UK
dATP nucleotide	Promega, Southampton UK
dCTP nucleotide	Promega, Southampton UK
dGTP nucleotide	Promega, Southampton UK
DIG Luminescent Detection Kit	Roche Diagnostics, Lewes UK
dTTP nucleotide	Promega, Southampton UK
Escherichia coli lipopolysaccharide	Sigma, Poole UK
Ethidium bromide	Sigma, Poole UK
Haematological indices	Sysmey NE8000, Toa Medical Electronics, Japan
Hybaid Omn-E thermal cycler	Hybaid, Teddington, UK
IL1β DNA primers	Sigma-Genosys Ltd, Cambridge UK
IL1β ELISA	R&D Systems Europe, Abingdon, UK
IL1B primers	Sigma-Genosys Ltd, Cambridge UK
IL1RA ELISA	R&D Systems Europe, Abingdon, UK
IL1RN primers	Oswell DNA Service, Southampton UK
IL6 ELISA	CLB, Amsterdam Holland
Intestinal permeability sugar probes	Ninewells Pharmaceuticals, Dundee UK
Jevity® enteral nutrition	Abbott, Berkshire UK
Lipopolysaccharide - Escherichia coli 0127:B8	Sigma, Poole UK
Monovette® blood tubes	Sarstedt, Numbrecht Germany
Nco1 endonuclease	Appligene-Oncor, Watford UK
NuSieve agarose	FMC Bioproducts, Rockland, Maine, USA
Nylon membrane	Roche Diagnostics, Lewes UK
Polaroid Polapan 665 and 667 film	Edinburgh Camera, Edinburgh UK
Polyacrylamide gel	Biorad, Hemel Hempstead, UK
Polymerase chain reaction reagents	Promega, Southampton UK
Puregene® DNA isolation kit	Gentra systems, North Carolina, USA
Roswell Park Memorial Institute medium 1640	Life Technologies, Paisley, UK
Serum CRP fluorometric immunoassay	Abbott, Berkshire UK
Soluble E-selectin ELISA	R&D Systems, Abingdon UK
Soluble P-selectin ELISA	R&D Systems, Abingdon UK

Southern blot hybridisation kit	Sigma, Poole UK
Statview 5.0 statistical software	SAS Institute, North Carolina, USA
Soluble TNFR-I ELISA	R&D Systems, Abingdon UK
Taq polymerase	Promega, Southampton UK
Taq1 endonuclease	Appligene-Oncor, Watford UK
TNFα ELISA	CLB, Amsterdam The Netherlands
TNF-308 primers	Sigma-Genosys Ltd, Cambridge UK
TNFB primers	Oswell DNA Service, Southampton UK
TNFB probe	Oswell DNA Service, Southampton UK
Urine urea measurement	Quintiles, Edinburgh UK

References

1. Bradley ELd. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. Arch Surg 1993; 128(5):586-90.
2. McKay CJ, Evans S, Sinclair M, et al. High early mortality rate from acute pancreatitis in Scotland, 1984- 1995 [see comments]. Br J Surg 1999; 86(10):1302-5.
3. de Beaux AC, Palmer KR, Carter DC. Factors influencing morbidity and mortality in acute pancreatitis; an analysis of 279 cases. Gut 1995; 37(1):121-6.
4. Fenton-Lee D, Imrie CW. Pancreatic necrosis: assessment of outcome related to quality of life and cost of management [see comments]. Br J Surg 1993; 80(12):1579-82.
5. Appelros S, Borgstrom A. Incidence, aetiology and mortality rate of acute pancreatitis over 10 years in a defined urban population in Sweden. Br J Surg 1999; 86(4):465-70.
6. Halvorsen FA, Ritland S. Acute pancreatitis in Buskerud County, Norway. Incidence and etiology. Scand J Gastroenterol 1996; 31(4):411-4.
7. Jaakkola M, Nordback I. Pancreatitis in Finland between 1970 and 1989. Gut 1993; 34(9):1255-60.
8. Wilson C, Imrie CW. Changing patterns of incidence and mortality from acute pancreatitis in Scotland, 1961-1985. Br J Surg 1990; 77(7):731-4.
9. Corfield AP, Cooper MJ, Williamson RC. Acute pancreatitis: a lethal disease of increasing incidence. Gut 1985; 26(7):724-9.
10. Trapnell JE, Duncan EH. Patterns of incidence in acute pancreatitis. Br Med J 1975; 2(5964):179-83.
11. Giggs JA, Bourke JB, Katschinski B. The epidemiology of primary acute pancreatitis in Greater Nottingham: 1969-1983. Soc Sci Med 1988; 26(1):79-89.
12. De Bolla AR, Obeid ML. Mortality in acute pancreatitis. Ann R Coll Surg Engl 1984; 66(3):184-6.
13. Lankisch PG, Schirren CA, Schmidt H, et al. Etiology and incidence of acute pancreatitis: a 20-year study in a single institution. Digestion 1989; 44(1):20-5.
14. Glazer G, Mann DV. United Kingdom guidelines for the management of acute pancreatitis. Gut 1998; 42(suppl 2):S1-S13.
15. Lerch MM, Baas JC, Senninger N. The role of bile in acute pancreatitis. In Buchler MW, Uhl W, Friess H, Malfertheiner P, eds. Acute pancreatitis: Current concepts in biology and therapy. Berlin: Blackwell Wissenschafts-Verlag, 1999. pp. 89-96.
16. DiMagno EP, Shorter RG, Taylor WF, Go VL. Relationships between pancreaticobiliary ductal anatomy and pancreatic ductal and parenchymal histology. Cancer 1982; 49(2):361-8.
17. Carr-Locke DL, Gregg JA. Endoscopic manometry of pancreatic and biliary sphincter zones in man. Basal results in healthy volunteers. Dig Dis Sci 1981; 26(1):7-15.
18. Menguy RB, Hallenback GA, Bollman JL, Grindlay JH. Intraductal pressures and sphincteric resistance in canine pancreatic and biliary ducts after various stimuli. Surg Gynecol Obstet 1958; 106:306-20.
19. Widdison AL, Alvarez C, Reber HA. The low-pressure duct perfusion model of acute pancreatitis. Eur Surg Res 1992; 24(Suppl 1):55-61.
20. Aho HJ, Koskensalo SM, Nevalainen TJ. Experimental pancreatitis in the rat. Sodium taurocholate-induced acute haemorrhagic pancreatitis. Scand J Gastroenterol 1980; 15(4):411-6.

21. Robinson TM, Dunphy JE. Continuous perfusion of bile and protease activators through the pancreas. JAMA 1963(183):530-3.

22. White TT, Magee DF. Perfusion of the dog pancreas with bile without production of acute pancreatitis. Ann Surg 1960; 151:245-50.

23. Dreiling DA, Bordalo OR, Noronha M, Greenstein R. Update: Big duct, little duct or toxi metabolic pathogenesis of pancreatitis. Am J Gastroenterol 1979; 71(4):424-6.

24. Pitchumoni CS, Bordalo O. Evaluation of hypotheses on pathogenesis of alcoholic pancreatitis. Am J Gastroenterol 1996; 91(4):637-47.

25. Sarles H, Sahel J. Pathology of chronic calcifying pancreatitis. Am J Gastroenterol 1976; 66(2):117-39.

26. Sahel J, Sarles H. Modifications of pure human pancreatic juice induced by chronic alcohol consumption. Dig Dis Sci 1979; 24(12):897-905.

27. Multigner L, Figarella C, Sarles H. Diagnosis of chronic pancreatitis by measurement of lactoferrin in duodenal juice. Gut 1981; 22(5):350-4.

28. Nordback IH, MacGowan S, Potter JJ, Cameron JL. The role of acetaldehyde in the pathogenesis of acute alcoholic pancreatitis. Ann Surg 1991; 214(6):671-8.

29. Mithofer K, Fernandez-del Castillo C, Rattner D, Warshaw AL. Subcellular kinetics of early trypsinogen activation in acute rodent pancreatitis. Am J Physiol 1998; 274(1 Pt 1):G71-9.

30. Frick TW, Fernandez-del Castillo C, Bimmler D, Warshaw AL. Elevated calcium and activation of trypsinogen in rat pancreatic acini. Gut 1997; 41(3):339-43.

31. Zhou W, Shen F, Miller JE, et al. Evidence for altered cellular calcium in the pathogenetic mechanism of acute pancreatitis in rats. J Surg Res 1996; 60(1):147-55.

32. Ward JB, Petersen OH, Jenkins SA, Sutton R. Is an elevated concentration of acinar cytosolic free ionised calcium the trigger for acute pancreatitis? Lancet 1995; 346(8981):1016-9.

33. Laskowski M, Ikunoshin K. Protein inhibitors of proteinases. Annu Rev Biochem 1980; 49:593-626.

34. Whitcomb DC, Gorry MC, Preston RA, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 1996; 14(2):141-5.

35. Scholmerich J, Schumichen C, Lausen M, et al. Scintigraphic assessment of leukocyte infiltration in acute pancreatitis using technetium-99m-hexamethyl propylene amine oxine as leukocyte label. Dig Dis Sci 1991; 36(1):65-70.

36. Werner J, Dragotakes SC, Fernandez-del Castillo C, et al. Technetium-99m-labeled white blood cells: a new method to define the local and systemic role of leukocytes in acute experimental pancreatitis. Ann Surg 1998; 227(1):86-94.

37. Sanfey H, Cameron JL. Increased capillary permeability: an early lesion in acute pancreatitis. Surgery 1984; 96(3):485-91.

38. Sweiry JH, Mann GE. Pancreatic microvascular permeability in caerulein-induced acute pancreatitis. Am J Physiol 1991; 261(4 Pt 1):G685-92.

39. Vaccaro MI, Ropolo A, Grasso D, et al. Pancreatic acinar cells submitted to stress activate TNF-alpha gene expression. Biochem Biophys Res Commun 2000; 268(2):485-90.

40. Grady T, Liang P, Ernst SA, Logsdon CD. Chemokine gene expression in rat pancreatic acinar cells is an early event associated with acute pancreatitis. Gastroenterology 1997; 113(6):1966-75.

41. Imrie CW, Benjamin IS, Ferguson JC, et al. A single-centre double-blind trial of Trasylol therapy in primary acute pancreatitis. Br J Surg 1978; 65(5):337-41.

42. Rinderknecht H. Fatal pancreatitis, a consequence of excessive leukocyte stimulation? Int J Pancreatol 1988; 3(2-3):105-12.

43. Border JR. Multiple systems organ failure [editorial; comment] [see comments]. Ann Surg 1992; 216(2):111-6.

44. Carswell EA, Old LJ, Kassel RL, et al. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A 1975; 72(9):3666-70.

45. Pennica D, Nedwin GE, Hayflick JS, et al. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature 1984; 312(5996):724-9.

46. Shirai T, Yamaguchi H, Ito H, et al. Cloning and expression in Escherichia coli of the gene for human tumour necrosis factor. Nature 1985; 313(6005):803-6.

47. Wang AM, Creasey AA, Ladner MB, et al. Molecular cloning of the complementary DNA for human tumor necrosis factor. Science 1985; 228(4696):149-54.

48. Moss ML, Jin SL, Milla ME, et al. Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha [published erratum appears in Nature 1997 Apr 17;386(6626):738]. Nature 1997; 385(6618):733-6.

49. Black RA, Rauch CT, Kozlosky CJ, et al. A metalloproteinase disintegrin that releases tumour-necrosis factor- alpha from cells. Nature 1997; 385(6618):729-33.

50. Beutler BA, Milsark IW, Cerami A. Cachectin/tumor necrosis factor: production, distribution, and metabolic fate in vivo. J Immunol 1985; 135(6):3972-7.

51. Flick DA, Gifford GE. Pharmacokinetics of murine tumor necrosis factor. J Immunopharmacol 1986; 8(1):89-97.

52. Decoster E, Vanhaesebroeck B, Vandenabeele P, et al. Generation and biological characterization of membrane-bound, uncleavable murine tumor necrosis factor. J Biol Chem 1995; 270(31):18473-8.

53. Smith RA, Baglioni C. The active form of tumor necrosis factor is a trimer. J Biol Chem 1987; 262(15):6951-4.

54. Gray PW, Aggarwal BB, Benton CV, et al. Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumour necrosis activity. Nature 1984; 312(5996):721-4.

55. Li CB, Gray PW, Lin PF, et al. Cloning and expression of murine lymphotoxin cDNA. J Immunol 1987; 138(12):4496-501.

56. Ware CF, Crowe PD, Grayson MH, et al. Expression of surface lymphotoxin and tumor necrosis factor on activated T, B, and natural killer cells. J Immunol 1992; 149(12):3881-8.

57. Hochman PS, Majeau GR, Mackay F, Browning JL. Proinflammatory responses are efficiently induced by homotrimeric but not heterotrimeric lymphotoxin ligands. J Inflamm 1995; 46(4):220-34.

58. Zhang M, Tracey KJ. Tumor necrosis factor. In Thomson A, ed. The Cytokine Handbook. London: Academic Press, 1998. pp. 517-48.

59. Tracey KJ, Cerami A. Tumor necrosis factor: an updated review of its biology. Crit Care Med 1993; 21(10 Suppl):S415-22.

60. Hesse DG, Tracey KJ, Fong Y, et al. Cytokine appearance in human endotoxemia and primate bacteremia. Surg Gynecol Obstet 1988; 166(2):147-53.

61. Tracey KJ, Lowry SF, Fahey TJd, et al. Cachectin/tumor necrosis factor induces lethal shock and stress hormone responses in the dog. Surg Gynecol Obstet 1987; 164(5):415-22.

62. Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature 1987; 330(6149):662-4.

63. Mohler KM, Torrance DS, Smith CA, et al. Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. J Immunol 1993; 151(3):1548-61.

64. van Deventer SJ, Buller HR, ten Cate JW, et al. Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. Blood 1990; 76(12):2520-6.

65. Michie HR, Manogue KR, Spriggs DR, et al. Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med 1988; 318(23):1481-6.

66. van der Poll T, van Deventer SJ, ten Cate H, et al. Tumor necrosis factor is involved in the appearance of interleukin-1 receptor antagonist in endotoxemia. J Infect Dis 1994; 169(3):665-7.

67. van Deventer SJ, Hart M, van der Poll T, et al. Endotoxin and tumor necrosis factor-alpha-induced interleukin-8 release in humans. J Infect Dis 1993; 167(2):461-54.

68. Michie HR, Spriggs DR, Manogue KR, et al. Tumor necrosis factor and endotoxin induce similar metabolic responses in human beings. Surgery 1988; 104(2):280-6.

69. Goldie AS, Fearon KC, Ross JA, et al. Natural cytokine antagonists and endogenous antiendotoxin core antibodies in sepsis syndrome. The Sepsis Intervention Group. Jama 1995; 274(2):172-7.

70. Abraham E, Anzueto A, Gutierrez G, et al. Double-blind randomised controlled trial of monoclonal antibody to human tumour necrosis factor in treatment of septic shock. NORASEPT II Study Group [see comments]. Lancet 1998; 351(9107):929-33.

71. Abraham E, Wunderink R, Silverman H, et al. Efficacy and safety of monoclonal antibody to human tumor necrosis factor alpha in patients with sepsis syndrome. A randomized, controlled, double-blind, multicenter clinical trial. TNF-alpha MAb Sepsis Study Group. Jama 1995; 273(12):934-41.

72. Reinhart K, Wiegand-Lohnert C, Grimminger F, et al. Assessment of the safety and efficacy of the monoclonal anti-tumor necrosis factor antibody-fragment, MAK 195F, in patients with sepsis and septic shock: a multicenter, randomized, placebo-controlled, dose-ranging study [see comments] [published erratum appears in Crit Care Med 1996 Sep;24(9):1608]. Crit Care Med 1996; 24(5):733-42.

73. Abraham E, Glauser MP, Butler T, et al. p55 Tumor necrosis factor receptor fusion protein in the treatment of patients with severe sepsis and septic shock. A randomized controlled multicenter trial. Ro 45-2081 Study Group. Jama 1997; 277(19):1531-8.

74. Fisher CJ, Jr., Agosti JM, Opal SM, et al. Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group [see comments]. N Engl J Med 1996; 334(26):1697-702.

75. Cohen J, Carlet J. INTERSEPT: an international, multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor-alpha in patients with sepsis. International Sepsis Trial Study Group. Crit Care Med 1996; 24(9):1431-40.

76. Bone RC. Sir Isaac Newton, sepsis, SIRS, and CARS. Crit Care Med 1996; 24(7):1125-8.

77. Norman JG, Fink GW, Denham W, et al. Tissue-specific cytokine production during experimental acute pancreatitis. A probable mechanism for distant organ dysfunction. Dig Dis Sci 1997; 42(8):1783-8.

78. Hughes CB, Gaber LW, Kotb M, et al. Induction of acute pancreatitis in germ-free rats: evidence of a primary role for tumor necrosis factor-alpha. Surgery 1995; 117(2):201-5.

79. Wang XD, Borjesson A, Sun ZW, et al. The association of type II pneumocytes and endothelial permeability with the pulmonary custocyte system in experimental acute pancreatitis. Eur J Clin Invest 1998; 28(9):778-85.

80. Van Laethem JL, Eskinazi R, Louis H, et al. Multisystemic production of interleukin 10 limits the severity of acute pancreatitis in mice. Gut 1998; 43(3):408-13.

81. Yang J, Murphy C, Denham W, et al. Evidence of a central role for p38 map kinase induction of tumor necrosis factor alpha in pancreatitis-associated pulmonary injury. Surgery 1999; 126(2):216-22.

82. Dunn JA, Li C, Ha T, et al. Therapeutic modification of nuclear factor kappa B binding activity and tumor necrosis factor-alpha gene expression during acute biliary pancreatitis. Am Surg 1997; 63(12):1036-43; discussion 1043-4.

83. Denham W, Fink G, Yang J, et al. Small molecule inhibition of tumor necrosis factor gene processing during acute pancreatitis prevents cytokine cascade progression and attenuates pancreatitis severity. Am Surg 1997; 63(12):1045-9; discussion 1049-50.

84. Guice KS, Oldham KT, Remick DG, et al. Anti-tumor necrosis factor antibody augments edema formation in caerulein-induced acute pancreatitis. J Surg Res 1991; 51(6):495-9.

85. Grewal HP, Mohey el Din A, Gaber L, et al. Amelioration of the physiologic and biochemical changes of acute pancreatitis using an anti-TNF-alpha polyclonal antibody. Am J Surg 1994; 167(1):214-8; discussion 218-9.

86. Hughes CB, Grewal HP, Gaber LW, et al. Anti-TNFalpha therapy improves survival and ameliorates the pathophysiologic sequelae in acute pancreatitis in the rat. Am J Surg 1996; 171(2):274-80.

87. Norman JG, Fink GW, Messina J, et al. Timing of tumor necrosis factor antagonism is critical in determining outcome in murine lethal acute pancreatitis. Surgery 1996; 120(3):515-21.

88. Denham W, Yang J, Fink G, et al. Gene targeting demonstrates additive detrimental effects of interleukin 1 and tumor necrosis factor during pancreatitis. Gastroenterology 1997; 113(5):1741-6.

89. Brivet FG, Emilie D, Galanaud P. Pro- and anti-inflammatory cytokines during acute severe pancreatitis: an early and sustained response, although unpredictable of death. Parisian Study Group on Acute Pancreatitis [see comments]. Crit Care Med 1999; 27(4):749-55.

90. Paajanen H, Laato M, Jaakkola M, et al. Serum tumour necrosis factor compared with C-reactive protein in the early assessment of severity of acute pancreatitis. Br J Surg 1995; 82(2):271-3.

91. de Beaux AC, Goldie AS, Ross JA, et al. Serum concentrations of inflammatory mediators related to organ failure in patients with acute pancreatitis. Br J Surg 1996; 83(3):349-53.

92. Exley AR, Leese T, Holliday MP, et al. Endotoxaemia and serum tumour necrosis factor as prognostic markers in severe acute pancreatitis. Gut 1992; 33(8):1126-8.

93. Banks RE, Evans SW, Alexander D, et al. Is fatal pancreatitis a consequence of excessive leukocyte stimulation? The role of tumor necrosis factor alpha. Cytokine 1991; 3(1):12-6.

94. Grewal HP, Kotb M, el Din AM, et al. Induction of tumor necrosis factor in severe acute pancreatitis and its subsequent reduction after hepatic passage. Surgery 1994; 115(2):213-21.

95. Kaufmann P, Tilz GP, Lueger A, Demel U. Elevated plasma levels of soluble tumor necrosis factor receptor (sTNFRp60) reflect severity of acute pancreatitis [see comments]. Intensive Care Med 1997; 23(8):841-8.

96. Soong CV, Lewis HG, Halliday MI, Rowlands BJ. Intramucosal acidosis and the inflammatory response in acute pancreatitis. Am J Gastroenterol 1999; 94(9):2423-9.

97. Spinas GA, Keller U, Brockhaus M. Release of soluble receptors for tumor necrosis factor (TNF) in relation to circulating TNF during experimental endotoxinemia. J Clin Invest 1992; 90(2):533-6.

98. Lantz M, Malik S, Slevin ML, Olsson I. Infusion of tumor necrosis factor (TNF) causes an increase in circulating TNF-binding protein in humans. Cytokine 1990; 2(6):402-6.

99. McKay CJ, Gallagher G, Brooks B, et al. Increased monocyte cytokine production in association with systemic complications in acute pancreatitis. Br J Surg 1996; 83(7):919-23.

100. de Beaux AC, Ross JA, Maingay JP, et al. Proinflammatory cytokine release by peripheral blood mononuclear cells from patients with acute pancreatitis. Br J Surg 1996; 83(8):1071-5.

101. van der Poll T, Calvano SE, Kumar A, et al. Endotoxin induces downregulation of tumor necrosis factor receptors on circulating monocytes and granulocytes in humans. Blood 1995; 86(7):2754-9.

102. Paleolog EM, Delasalle SA, Buurman WA, Feldmann M. Functional activities of receptors for tumor necrosis factor-alpha on human vascular endothelial cells. Blood 1994; 84(8):2578-90.

103. Spengler U, Zachoval R, Gallati H, et al. Serum levels and in situ expression of TNF-alpha and TNF-alpha binding proteins in inflammatory liver diseases. Cytokine 1996; 8(11):864-72.

104. Lien E, Liabakk NB, Johnsen AC, et al. Polymorphonuclear granulocytes enhance lipopolysaccharide-induced soluble p75 tumor necrosis factor receptor release from mononuclear cells. Eur J Immunol 1995; 25(9):2714-7.

105. Galve de Rochemonteix B, Nicod LP, Dayer JM. Tumor necrosis factor soluble receptor 75: the principal receptor form released by human alveolar macrophages and monocytes in the presence of interferon gamma. Am J Respir Cell Mol Biol 1996; 14(3):279-87.

106. Bradley JR, Thiru S, Pober JS. Disparate localization of 55-kd and 75-kd tumor necrosis factor receptors in human endothelial cells. Am J Pathol 1995; 146(1):27-32.

107. Medvedev AE, Sundan A, Espevik T. Involvement of the tumor necrosis factor receptor p75 in mediating cytotoxicity and gene regulating activities. Eur J Immunol 1994; 24(11):2842-9.

108. Wang B, Kondo S, Shivji GM, et al. Tumour necrosis factor receptor II (p75) signalling is required for the migration of Langerhans' cells. Immunology 1996; 88(2):284-8.

109. Grell M. Tumor necrosis factor (TNF) receptors in cellular signaling of soluble and membrane-expressed TNF. J Inflamm 1995; 47(1-2):8-17.

110. Bjornberg F, Lantz M, Gullberg U. Metalloproteases and serineproteases are involved in the cleavage of the two tumour necrosis factor (TNF) receptors to soluble forms in the myeloid cell lines U-937 and THP-1. Scand J Immunol 1995; 42(4):418-24.

111. Ashkenazi A, Marsters SA, Capon DJ, et al. Protection against endotoxic shock by a tumor necrosis factor receptor immunoadhesin. Proc Natl Acad Sci U S A 1991; 88(23):10535-9.

112. Higuchi M, Aggarwal BB. Inhibition of ligand binding and antiproliferative effects of tumor necrosis factor and lymphotoxin by soluble forms of recombinant P60 and P80 receptors. Biochem Biophys Res Commun 1992; 182(2):638-43.

113. Aderka D, Engelmann H, Maor Y, et al. Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J Exp Med 1992; 175(2):323-9.

114. Calvano SE, van der Poll T, Coyle SM, et al. Monocyte tumor necrosis factor receptor levels as a predictor of risk in human sepsis. Arch Surg 1996; 131(4):434-7.

115. Schroder J, Stuber F, Gallati H, et al. Pattern of soluble TNF receptors I and II in sepsis. Infection 1995; 23(3):143-8.

116. March CJ, Mosley B, Larsen A, et al. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. Nature 1985; 315(6021):641-7.

117. Stewart RJ, Marsden PA. Biologic control of the tumor necrosis factor and interleukin-1 signaling cascade. Am J Kidney Dis 1995; 25(6):954-66.

118. Colotta F, Re F, Muzio M, et al. Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. Science 1993; 261(5120):472-5.

119. Lonnemann G, Endres S, Van der Meer JW, et al. Differences in the synthesis and kinetics of release of interleukin 1 alpha, interleukin 1 beta and tumor necrosis factor from human mononuclear cells. Eur J Immunol 1989; 19(9):1531-6.

120. Schindler R, Mancilla J, Endres S, et al. Correlations and interactions in the production of interleukin-6 (IL- 6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. Blood 1990; 75(1):40-7.

121. Dinarello CA. Biologic basis for interleukin-1 in disease. Blood 1996; 87(6):2095-147.

122. Roux-Lombard P. The interleukin-1 family. Eur Cytokine Netw 1998; 9(4):565-76.

123. Eisenberg SP, Evans RJ, Arend WP, et al. Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. Nature 1990; 343(6256):341-6.

124. Carter DB, Deibel MR, Jr., Dunn CJ, et al. Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein [see comments]. Nature 1990; 344(6267):633-8.

125. Hannum CH, Wilcox CJ, Arend WP, et al. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 1990; 343(6256):336-40.

126. Aiura K, Gelfand JA, Burke JF, et al. Interleukin-1 (IL-1) receptor antagonist prevents Staphylococcus epidermidis-induced hypotension and reduces circulating levels of tumor necrosis factor and IL-1 beta in rabbits. Infect Immun 1993; 61(8):3342-50.

127. Fischer E, Marano MA, Van Zee KJ, et al. Interleukin-1 receptor blockade improves survival and hemodynamic performance in Escherichia coli septic shock, but fails to alter host responses to sublethal endotoxemia. J Clin Invest 1992; 89(5):1551-7.

128. Ohlsson K, Bjork P, Bergenfeldt M, et al. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. Nature 1990; 348(6301):550-2.

129. Wakabayashi G, Gelfand JA, Burke JF, et al. A specific receptor antagonist for interleukin 1 prevents Escherichia coli-induced shock in rabbits. Faseb J 1991; 5(3):338-43.

130. Opal SM, Fisher CJ, Jr., Dhainaut JF, et al. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group [see comments]. Crit Care Med 1997; 25(7):1115-24.

131. Fink GW, Norman JG. Intrapancreatic interleukin-1beta gene expression by specific leukocyte populations during acute pancreatitis. J Surg Res 1996; 63(1):369-73.

132. Fink GW, Norman JG. Specific changes in the pancreatic expression of the interleukin 1 family of genes during experimental acute pancreatitis. Cytokine 1997; 9(12):1023-7.

133. Norman J, Yang J, Fink G, et al. Severity and mortality of experimental pancreatitis are dependent on interleukin-1 converting enzyme (ICE). J Interferon Cytokine Res 1997; 17(2):113-8.

134. Norman JG, Fink G, Franz M, et al. Active interleukin-1 receptor required for maximal progression of acute pancreatitis. Ann Surg 1996; 223(2):163-9.

135. Norman JG, Fink GW, Sexton C, Carter G. Transgenic animals demonstrate a role for the IL-1 receptor in regulating IL-1beta gene expression at steady-state and during the systemic stress induced by acute pancreatitis. J Surg Res 1996; 63(1):231-6.

136. Tanaka N, Murata A, Uda K, et al. Interleukin-1 receptor antagonist modifies the changes in vital organs induced by acute necrotizing pancreatitis in a rat experimental model. Crit Care Med 1995; 23(5):901-8.

137. Norman J, Franz M, Messina J, et al. Interleukin-1 receptor antagonist decreases severity of experimental acute pancreatitis. Surgery 1995; 117(6):648-55.

138. Norman JG, Franz MG, Fink GS, et al. Decreased mortality of severe acute pancreatitis after proximal cytokine blockade. Ann Surg 1995; 221(6):625-31; discussion 631-4.

139. Biffl WL, Moore EE, Moore FA, Peterson VM. Interleukin-6 in the injured patient. Marker of injury or mediator of inflammation? Ann Surg 1996; 224(5):647-64.

140. Tilg H, Dinarello CA, Mier JW. IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. Immunol Today 1997; 18(9):428-32.

141. Tilg H, Trehu E, Atkins MB, et al. Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. Blood 1994; 83(1):113-8.

142. Simms HH, D'Amico R. Polymorphonuclear leukocyte dysregulation during the systemic inflammatory response syndrome. Blood 1994; 83(5):1398-407.

143. Leser HG, Gross V, Scheibenbogen C, et al. Elevation of serum interleukin-6 concentration precedes acute-phase response and reflects severity in acute pancreatitis. Gastroenterology 1991; 101(3):782-5.

144. Viedma JA, Perez-Mateo M, Dominguez JE, Carballo F. Role of interleukin-6 in acute pancreatitis. Comparison with C-reactive protein and phospholipase A. Gut 1992; 33(9):1264-7.

145. Berney T, Gasche Y, Robert J, et al. Serum profiles of interleukin-6, interleukin-8, and interleukin-10 in patients with severe and mild acute pancreatitis. Pancreas 1999; 18(4):371-7.

146. Pezzilli R, Billi P, Miniero R, et al. Serum interleukin-6, interleukin-8, and beta 2-microglobulin in early assessment of severity of acute pancreatitis. Comparison with serum C- reactive protein. Dig Dis Sci 1995; 40(11):2341-8.

147. Heath DI, Cruickshank A, Gudgeon M, et al. Role of interleukin-6 in mediating the acute phase protein response and potential as an early means of severity assessment in acute pancreatitis. Gut 1993; 34(1):41-5.

148. Adams DH, Lloyd AR. Chemokines: leucocyte recruitment and activation cytokines. Lancet 1997; 349(9050):490-5.

149. Gross V, Andreesen R, Leser HG, et al. Interleukin-8 and neutrophil activation in acute pancreatitis. Eur J Clin Invest 1992; 22(3):200-3.

150. Chen CC, Wang SS, Lee FY, et al. Proinflammatory cytokines in early assessment of the prognosis of acute pancreatitis. Am J Gastroenterol 1999; 94(1):213-8.

151. Hynninen M, Valtonen M, Markkanen H, et al. Intramucosal pH and endotoxin and cytokine release in severe acute pancreatitis. Shock 2000; 13(1):79-82.

152. Osman MO, Kristensen JU, Jacobsen NO, et al. A monoclonal anti-interleukin 8 antibody (WS-4) inhibits cytokine response and acute lung injury in experimental severe acute necrotising pancreatitis in rabbits. Gut 1998; 43(2):232-9.

153. Emanuelli G, Montrucchio G, Gaia E, et al. Experimental acute pancreatitis induced by platelet activating factor in rabbits. Am J Pathol 1989; 134(2):315-26.

154. Kald B, Kald A, Ihse I, Tagesson C. Release of platelet-activating factor in acute experimental pancreatitis. Pancreas 1993; 8(4):440-2.

155. Zhou W, McCollum MO, Levine BA, Olson MS. Role of platelet-activating factor in pancreatitis-associated acute lung injury in the rat. Am J Pathol 1992; 140(4):971-9.

156. Dabrowski A, Gabryelewicz A, Chyczewski L. The effect of platelet activating factor antagonist (BN 52021) on cerulein-induced acute pancreatitis with reference to oxygen radicals. Int J Pancreatol 1991; 8(1):1-11.

157. Konturek SJ, Dembinski A, Konturek PJ, et al. Role of platelet activating factor in pathogenesis of acute pancreatitis in rats. Gut 1992; 33(9):1268-74.

158. Formela LJ, Wood LM, Whittaker M, Kingsnorth AN. Amelioration of experimental acute pancreatitis with a potent platelet- activating factor antagonist. Br J Surg 1994; 81(12):1783-5.

159. Galloway SW, Kingsnorth AN. Lung injury in the microembolic model of acute pancreatitis and amelioration by lexipafant (BB-882), a platelet-activating factor antagonist. Pancreas 1996; 13(2):140-6.

160. Andersson R, Wang X, Sun Z, et al. Effect of a platelet-activating factor antagonist on pancreatitis- associated gut barrier dysfunction in rats. Pancreas 1998; 17(2):107-19.

161. Leonhardt U, Fayyazzi A, Seidensticker F, et al. Influence of a platelet-activating factor antagonist on severe pancreatitis in two experimental models. Int J Pancreatol 1992; 12(2):161-6.

162. Hofbauer B, Saluja AK, Bhatia M, et al. Effect of recombinant platelet-activating factor acetylhydrolase on two models of experimental acute pancreatitis. Gastroenterology 1998; 115(5):1238-47.

163. Kingsnorth AN, Galloway SW, Formela LJ. Randomized, double-blind phase II trial of Lexipafant, a platelet- activating factor antagonist, in human acute pancreatitis. Br J Surg 1995; 82(10):1414-20.

164. McKay C, Baxter J, Imrie C. A randomized, controlled trial of octreotide in the management of patients with acute pancreatitis. Int J Pancreatol 1997; 21(1):13-9.

165. Kingsnorth AN. Early treatment with lexipafant, a platelet activating factor antagonist, reduces mortality in acute pancreatitis: a double blind, randomized, placebo controlled study. Gastroenterology 1997; 112:A452.

166. Van Laethem JL, Marchant A, Delvaux A, et al. Interleukin 10 prevents necrosis in murine experimental acute pancreatitis. Gastroenterology 1995; 108(6):1917-22.

167. Rongione AJ, Kusske AM, Reber HA, et al. Interleukin-10 Reduces Circulating Levels of Serum Cytokines in Experimental Pancreatitis. J Gastrointest Surg 1997; 1(2):159-166.

168. Osman MO, Jacobsen NO, Kristensen JU, et al. IT 9302, a synthetic interleukin-10 agonist, diminishes acute lung injury in rabbits with acute necrotizing pancreatitis. Surgery 1998; 124(3):584-92.

169. Rongione AJ, Kusske AM, Kwan K, et al. Interleukin 10 reduces the severity of acute pancreatitis in rats. Gastroenterology 1997; 112(3):960-7.

170. Denham W, Denham D, Yang J, et al. Transient human gene therapy: a novel cytokine regulatory strategy for experimental pancreatitis. Ann Surg 1998; 227(6):812-20.

171. Gloor B, Todd KE, Lane JS, et al. Mechanism of increased lung injury after acute pancreatitis in IL-10 knockout mice. J Surg Res 1998; 80(1):110-4.

172. Pezzilli R, Billi P, Miniero R, Barakat B. Serum interleukin-10 in human acute pancreatitis. Dig Dis Sci 1997; 42(7):1469-72.

173. Chen CC, Wang SS, Lu RH, et al. Serum interleukin 10 and interleukin 11 in patients with acute pancreatitis. Gut 1999; 45(6):895-9.

174. Simovic MO, Bonham MJ, Abu-Zidan FM, Windsor JA. Anti-inflammatory cytokine response and clinical outcome in acute pancreatitis. Crit Care Med 1999; 27(12):2662-5.

175. Barie PS, Tahamont MV, Malik AB. Prevention of increased pulmonary vascular permeability after pancreatitis by granulocyte depletion in sheep. Am Rev Respir Dis 1982; 126(5):904-8.

176. Bhatia M, Saluja AK, Hofbauer B, et al. The effects of neutrophil depletion on a completely noninvasive model of acute pancreatitis-associated lung injury. Int J Pancreatol 1998; 24(2):77-83.

177. Guice KS, Oldham KT, Caty MG, et al. Neutrophil-dependent, oxygen-radical mediated lung injury associated with acute pancreatitis. Ann Surg 1989; 210(6):740-7.

178. Frossard JL, Saluja A, Bhagat L, et al. The role of intercellular adhesion molecule 1 and neutrophils in acute pancreatitis and pancreatitis-associated lung injury. Gastroenterology 1999; 116(3):694-701.

179. Inoue S, Nakao A, Kishimoto W, et al. Anti-neutrophil antibody attenuates the severity of acute lung injury in rats with experimental acute pancreatitis. Arch Surg 1995; 130(1):93-8.

180. Sarr MG, Bulkley GB, Cameron JL. The role of leukocytes in the production of oxygen-derived free radicals in acute experimental pancreatitis. Surgery 1987; 101(3):292-6.

181. Papos M, Takacs T, Farkas G, et al. Prognostic role of 99mTc-HM-PAO-leukocyte scintigraphy in acute pancreatitis and in patients with pancreatic pseudocysts. Pancreas 1997; 14(1):9-15.

182. Liras G, Carballo F. An impaired phagocytic function is associated with leucocyte activation in the early stages of severe acute pancreatitis. Gut 1996; 39(1):39-42.

183. Widdison AL, Cunningham S. Immune function early in acute pancreatitis. Br J Surg 1996; 83(5):633-6.

184. Dominguez-Munoz JE, Carballo F, Garcia MJ, et al. Clinical usefulness of polymorphonuclear elastase in predicting the severity of acute pancreatitis: results of a multicentre study. Br J Surg 1991; 78(10):1230-4.

185. Larvin M, Alexander DJ, Switala SF, McMahon MJ. Impaired mononuclear phagocyte function in patients with severe acute pancreatitis: evidence from studies of plasma clearance of trypsin and monocyte phagocytosis. Dig Dis Sci 1993; 38(1):18-27.

186. Richter A, Nebe T, Wendl K, et al. HLA-DR expression in acute pancreatitis. Eur J Surg 1999; 165(10):947-51.

187. Pezzilli R, Billi P, Beltrandi E, et al. Circulating lymphocyte subsets in human acute pancreatitis. Pancreas 1995; 11(1):95-100.

188. Curley PJ, McMahon MJ, Lancaster F, et al. Reduction in circulating levels of CD4-positive lymphocytes in acute pancreatitis: relationship to endotoxin, interleukin 6 and disease severity [see comments]. Br J Surg 1993; 80(10):1312-5.

189. Menger MD, Vollmar B. Adhesion molecules as determinants of disease: from molecular biology to surgical research. Br J Surg 1996; 83(5):588-601.

190. Folch E, Salas A, Panes J, et al. Role of P-selectin and ICAM-1 in pancreatitis-induced lung inflammation in rats: significance of oxidative stress. Ann Surg 1999; 230(6):792-8; discussion 798-9.

191. Werner J, Z'Graggen K, Fernandez-del Castillo C, et al. Specific therapy for local and systemic complications of acute pancreatitis with monoclonal antibodies against ICAM-1. Ann Surg 1999; 229(6):834-40; discussion 841-2.

192. Lundberg AH, Fukatsu K, Gaber L, et al. Blocking Pulmonary ICAM-1 Expression Ameliorates Lung Injury in Established Diet-Induced Pancreatitis. Ann Surg 2001; 233(2):213-220.

193. Lundberg AH, Granger N, Russell J, et al. Temporal correlation of tumor necrosis factor-alpha release, upregulation of pulmonary ICAM-1 and VCAM-1, neutrophil sequestration, and lung injury in diet-induced pancreatitis. J Gastrointest Surg 2000; 4(3):248-57.

194. Poch B, Gansauge F, Rau B, et al. The role of polymorphonuclear leukocytes and oxygen-derived free radicals in experimental acute pancreatitis: mediators of local destruction and activators of inflammation. FEBS Lett 1999; 461(3):268-72.

195. Foitzik T, Eibl G, Buhr HJ. Therapy for microcirculatory disorders in severe acute pancreatitis: comparison of delayed therapy with ICAM-1 antibodies and a specific endothelin A receptor antagonist. J Gastrointest Surg 2000; 4(3):240-6; discussion 247.

196. Wang X, Sun Z, Borjesson A, Andersson R. Inhibition of platelet-activating factor, intercellular adhesion molecule 1 and platelet endothelial cell adhesion molecule 1 reduces

experimental pancreatitis-associated gut endothelial barrier dysfunction. Br J Surg 1999; 86(3):411-6.

197. Kaufmann P, Tilz GP, Smolle KH, et al. Increased plasma concentrations of circulating intercellular adhesion molecule-1 (cICAM-1) in patients with necrotizing pancreatitis. Immunobiology 1996; 195(2):209-19.

198. Kansas GS. Selectins and their ligands: current concepts and controversies. Blood 1996; 88(9):3259-87.

199. McEver RP, Beckstead JH, Moore KL, et al. GMP-140, a platelet alpha-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. J Clin Invest 1989; 84(1):92-9.

200. Foreman KE, Vaporciyan AA, Bonish BK, et al. C5a-induced expression of P-selectin in endothelial cells. J Clin Invest 1994; 94(3):1147-55.

201. Sanders WE, Wilson RW, Ballantyne CM, Beaudet AL. Molecular cloning and analysis of in vivo expression of murine P- selectin. Blood 1992; 80(3):795-800.

202. Wyble CW, Hynes KL, Kuchibhotla J, et al. TNF-alpha and IL-1 upregulate membrane-bound and soluble E-selectin through a common pathway. J Surg Res 1997; 73(2):107-12.

203. Bevilacqua MP, Stengelin S, Gimbrone MA, Seed B. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. Science 1989; 243(4895):1160-5.

204. von Asmuth EJ, Smeets EF, Ginsel LA, et al. Evidence for endocytosis of E-selectin in human endothelial cells. Eur J Immunol 1992; 22(10):2519-26.

205. Subramaniam M, Koedam JA, Wagner DD. Divergent fates of P- and E-selectins after their expression on the plasma membrane. Mol Biol Cell 1993; 4(8):791-801.

206. Pigott R, Dillon LP, Hemingway IH, Gearing AJ. Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. Biochem Biophys Res Commun 1992; 187(2):584-9.

207. Dunlop LC, Skinner MP, Bendall LJ, et al. Characterization of GMP-140 (P-selectin) as a circulating plasma protein. J Exp Med 1992; 175(4):1147-50.

208. Blann AD, Lip GY. Hypothesis: is soluble P-selectin a new marker of platelet activation? Atherosclerosis 1997; 128(2):135-8.

209. Blann A, Seigneur M. Soluble markers of endothelial cell function. Clin Hemorheol Microcirc 1997; 17(1):3-11.

210. Lundberg AH, Granger DN, Russell J, et al. Quantitative measurement of P- and E-selectin adhesion molecules in acute pancreatitis: correlation with distant organ injury. Ann Surg 2000; 231(2):213-22.

211. Wilson AG, di Giovine FS, Duff GW. Genetics of tumour necrosis factor-alpha in autoimmune, infectious, and neoplastic diseases. J Inflamm 1995; 45(1):1-12.

212. Wilson AG, di Giovine FS, Blakemore AI, Duff GW. Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. Hum Mol Genet 1992; 1(5):353.

213. Wilson AG, Symons JA, McDowell TL, et al. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A 1997; 94(7):3195-9.

214. Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. Mol Immunol 1997; 34(5):391-9.

215. Louis E, Franchimont D, Piron A, et al. Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. Clin Exp Immunol 1998; 113(3):401-6.

216. Brinkman BM, Zuijdeest D, Kaijzel EL, et al. Relevance of the tumor necrosis factor alpha (TNF alpha) -308 promoter polymorphism in TNF alpha gene regulation [see comments]. J Inflamm 1995; 46(1):32-41.

217. Kroeger KM, Steer JH, Joyce DA, Abraham LJ. Effects of stimulus and cell type on the expression of the -308 tumour necrosis factor promoter polymorphism. Cytokine 2000; 12(2):110-9.

218. Nadel S, Newport MJ, Booy R, Levin M. Variation in the tumor necrosis factor-alpha gene promoter region may be associated with death from meningococcal disease. J Infect Dis 1996; 174(4):878-80.

219. McGuire W, Hill AV, Allsopp CE, et al. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. Nature 1994; 371(6497):508-10.

220. Mira JP, Cariou A, Grall F, et al. Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study [see comments]. Jama 1999; 282(6):561-8.

221. D'Alfonso S, Richiardi PM. A polymorphic variation in a putative regulation box of the TNFA promoter region. Immunogenetics 1994; 39(2):150-4.

222. Kaluza W, Reuss E, Grossmann S, et al. Different transcriptional activity and in vitro TNF-alpha production in psoriasis patients carrying the TNF-alpha 238A promoter polymorphism. J Invest Dermatol 2000; 114(6):1180-3.

223. Pociot F, D'Alfonso S, Compasso S, et al. Functional analysis of a new polymorphism in the human TNF alpha gene promoter. Scand J Immunol 1995; 42(4):501-4.

224. Huizinga TW, Westendorp RG, Bollen EL, et al. TNF-alpha promoter polymorphisms, production and susceptibility to multiple sclerosis in different groups of patients. J Neuroimmunol 1997; 72(2):149-53.

225. Westendorp RG, Langermans JA, Huizinga TW, et al. Genetic influence on cytokine production and fatal meningococcal disease [published erratum appears in Lancet 1997 Mar 1;349(9052):656] [see comments]. Lancet 1997; 349(9046):170-3.

226. Grove J, Daly AK, Bassendine MF, Day CP. Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis [see comments]. Hepatology 1997; 26(1):143-6.

227. Hohler T, Kruger A, Gerken G, et al. Tumor necrosis factor alpha promoter polymorphism at position -238 is associated with chronic active hepatitis C infection. J Med Virol 1998; 54(3):173-7.

228. Hohler T, Kruger A, Gerken G, et al. A tumor necrosis factor-alpha (TNF-alpha) promoter polymorphism is associated with chronic hepatitis B infection. Clin Exp Immunol 1998; 111(3):579-82.

229. Pociot F, Molvig J, Wogensen L, et al. A tumour necrosis factor beta gene polymorphism in relation to monokine secretion and insulin-dependent diabetes mellitus. Scand J Immunol 1991; 33(1):37-49.

230. Messer G, Spengler U, Jung MC, et al. Polymorphic structure of the tumor necrosis factor (TNF) locus: an NcoI polymorphism in the first intron of the human TNF-beta gene correlates with a variant amino acid in position 26 and a reduced level of TNF- beta production. J Exp Med 1991; 173(1):209-19.

231. Molvig J, Pociot F, Baek L, et al. Monocyte function in IDDM patients and healthy individuals. Scand J Immunol 1990; 32(3):297-311.

232. Pociot F, Briant L, Jongeneel CV, et al. Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-alpha and TNF-beta by human mononuclear cells: a possible link to insulin- dependent diabetes mellitus. Eur J Immunol 1993; 23(1):224-31.

233. Stuber F, Petersen M, Bokelmann F, Schade U. A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor-alpha concentrations and outcome of patients with severe sepsis. Crit Care Med 1996; 24(3):381-4.

234. Majetschak M, Flohe S, Obertacke U, et al. Relation of a TNF gene polymorphism to severe sepsis in trauma patients. Ann Surg 1999; 230(2):207-14.

235. Udalova IA, Nedospasov SA, Webb GC, et al. Highly informative typing of the human TNF locus using six adjacent polymorphic markers. Genomics 1993; 16(1):180-6.

236. Partanen J, Koskimies S. Low degree of DNA polymorphism in the HLA-linked lymphotoxin (tumour necrosis factor beta) gene. Scand J Immunol 1988; 28(3):313-6.

237. Jongeneel CV, Briant L, Udalova IA, et al. Extensive genetic polymorphism in the human tumor necrosis factor region and relation to extended HLA haplotypes. Proc Natl Acad Sci U S A 1991; 88(21):9717-21.

238. Ferencik S, Lindemann M, Horsthemke B, Grosse-Wilde H. A new restriction fragment length polymorphism of the human TNF-B gene detected by AspHI digest. Eur J Immunogenet 1992; 19(6):425-30.

239. Webb AC, Collins KL, Auron PE, et al. Interleukin-1 gene (IL1) assigned to long arm of human chromosome 2. Lymphokine Res 1986; 5(2):77-85.

240. di Giovine FS, Takhsh E, Blakemore AI, Duff GW. Single base polymorphism at -511 in the human interleukin-1 beta gene (IL1 beta). Hum Mol Genet 1992; 1(6):450.

241. Hulkkonen J, Laippala P, Hurne M. A rare allele combination of the interleukin-1 gene complex is associated with high interleukin-1 beta plasma levels in healthy individuals. Eur Cytokine Netw 2000; 11(2):251-5.

242. Hurme M, Santtila S. IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. Eur J Immunol 1998; 28(8):2598-602.

243. Pociot F, Molvig J, Wogensen L, et al. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. Eur J Clin Invest 1992; 22(6):396-402.

244. Wilkinson RJ, Patel P, Llewelyn M, et al. Influence of polymorphism in the genes for the interleukin (IL)-1 receptor antagonist and IL-1beta on tuberculosis. J Exp Med 1999; 189(12):1863-74.

245. Bioque G, Crusius JB, Koutroubakis I, et al. Allelic polymorphism in IL-1 beta and IL-1 receptor antagonist (IL-1Ra) genes in inflammatory bowel disease. Clin Exp Immunol 1995; 102(2):379-83.

246. Heresbach D, Alizadeh M, Dabadie A, et al. Significance of interleukin-1beta and interleukin-1 receptor antagonist genetic polymorphism in inflammatory bowel diseases. Am J Gastroenterol 1997; 92(7):1164-9.

247. Kantarci OH, Atkinson EJ, Hebrink DD, et al. Association of two variants in IL-1beta and IL-1 receptor antagonist genes with multiple sclerosis. J Neuroimmunol 2000; 106(1-2):220-7.

248. Takamatsu M, Yamauchi M, Maezawa Y, et al. Genetic polymorphisms of interleukin-1beta in association with the development of alcoholic liver disease in Japanese patients. Am J Gastroenterol 2000; 95(5):1305-11.

249. Tarlow JK, Blakemore AI, Lennard A, et al. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. Hum Genet 1993; 91(4):403-4.

250. Danis VA, Millington M, Hyland VJ, Grennan D. Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. Clin Exp Immunol 1995; 99(2):303-10.

251. Fang XM, Schroder S, Hoeft A, Stuber F. Comparison of two polymorphisms of the interleukin-1 gene family: interleukin-1 receptor antagonist polymorphism contributes to susceptibility to severe sepsis [see comments]. Crit Care Med 1999; 27(7):1330-4.

252. Smithies AM, Sargen K, Demaine AG, Kingsnorth AN. Investigation of the interleukin 1 gene cluster and its association with acute pancreatitis. Pancreas 2000; 20(3):234-40.

253. Schrijver HM, Crusius JB, Uitdehaag BM, et al. Association of interleukin-1beta and interleukin-1 receptor antagonist genes with disease severity in MS. Neurology 1999; 52(3):595-9.

254. Mansfield JC, Holden H, Tarlow JK, et al. Novel genetic association between ulcerative colitis and the anti- inflammatory cytokine interleukin-1 receptor antagonist. Gastroenterology 1994; 106(3):637-42.

255. Blakemore AI, Tarlow JK, Cork MJ, et al. Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. Arthritis Rheum 1994; 37(9):1380-5.

256. Clay FE, Tarlow JK, Cork MJ, et al. Novel interleukin-1 receptor antagonist exon polymorphisms and their use in allele-specific mRNA assessment. Hum Genet 1996; 97(6):723-6.

257. Bailly S, di Giovine FS, Blakemore AI, Duff GW. Genetic polymorphism of human interleukin-1 alpha. Eur J Immunol 1993; 23(6):1240-5.

258. Todd S, Naylor SL. Dinucleotide repeat polymorphism in the human interleukin 1, alpha gene (IL1A). Nucleic Acids Res 1991; 19(13):3756.

259. van den Velden PA, Reitsma PH. Amino acid dimorphism in IL1A is detectable by PCR amplification. Hum Mol Genet 1993; 2(10):1753.

260. Zuliani G, Hobbs HH. A high frequency of length polymorphisms in repeated sequences adjacent to Alu sequences. Am J Hum Genet 1990; 46(5):963-9.

261. McDowell TL, Symons JA, Ploski R, et al. A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1 alpha polymorphism. Arthritis Rheum 1995; 38(2):221-8.

262. Sunden SL, Businga T, Beck J, et al. Chromosomal assignment of 2900 tri- and tetranucleotide repeat markers using NIGMS somatic cell hybrid panel 2. Genomics 1996; 32(1):15-20.

263. Spur NK, Bashir R, Bushby K, et al. Report and abstracts of the Fourth International Workshop on Human Chromosome 2 Mapping 1996. Cytogenet Cell Genet 1996; 73(4):255-73.

264. Deitch EA. Multiple organ failure. Pathophysiology and potential future therapy [see comments]. Ann Surg 1992; 216(2):117-34.

265. Sparwasser T, Miethke T, Lipford G, et al. Bacterial DNA causes septic shock [letter]. Nature 1997; 386(6623):336-7.

266. Bjarnason I, MacPherson A, Hollander D. Intestinal permeability: an overview [see comments]. Gastroenterology 1995; 108(5):1566-81.

267. Travis S, Menzies I. Intestinal permeability: functional assessment and significance [editorial]. Clin Sci (Colch) 1992; 82(5):471-88.

268. O'Dwyer ST, Michie HR, Ziegler TR, et al. A single dose of endotoxin increases intestinal permeability in healthy humans. Arch Surg 1988; 123(12):1459-64.

269. Johnston JD, Harvey CJ, Menzies IS, Treacher DF. Gastrointestinal permeability and absorptive capacity in sepsis. Crit Care Med 1996; 24(7):1144-9.

270. Harris CE, Griffiths RD, Freestone N, et al. Intestinal permeability in the critically ill. Intensive Care Med 1992; 18(1):38-41.

271. Ziegler TR, Smith RJ, O'Dwyer ST, et al. Increased intestinal permeability associated with infection in burn patients. Arch Surg 1988; 123(11):1313-9.

272. Thompson JS. The intestinal response to critical illness. Am J Gastroenterol 1995; 90(2):190-200.

273. Maxton DG, Menzies IS, Slavin B, Thompson RP. Small-intestinal function during enteral feeding and starvation in man. Clin Sci 1989; 77(4):401-6.

274. Buchman AL, Moukarzel AA, Bhuta S, et al. Parenteral nutrition is associated with intestinal morphologic and functional changes in humans. JPEN J Parenter Enteral Nutr 1995; 19(6):453-60.

275. Hadfield RJ, Sinclair DG, Houldsworth PE, Evans TW. Effects of enteral and parenteral nutrition on gut mucosal permeability in the critically ill. Am J Respir Crit Care Med 1995; 152(5 Pt 1):1545-8.

276. Carr CS, Ling KD, Boulos P, Singer M. Randomised trial of safety and efficacy of immediate postoperative enteral feeding in patients undergoing gastrointestinal resection [see comments]. Bmj 1996; 312(7035):869-71.

277. Brooks AD, Hochwald SN, Heslin MJ, et al. Intestinal permeability after early postoperative enteral nutrition in patients with upper gastrointestinal malignancy [see comments]. JPEN J Parenter Enteral Nutr 1999; 23(2):75-9.

278. Heslin MJ, Latkany L, Leung D, et al. A prospective, randomized trial of early enteral feeding after resection of upper gastrointestinal malignancy. Ann Surg 1997; 226(4):567-77; discussion 577-80.

279. Bower RH, Cerra FB, Bershadsky B, et al. Early enteral administration of a formula (Impact) supplemented with arginine, nucleotides, and fish oil in intensive care unit patients: results of a multicenter, prospective, randomized, clinical trial [see comments]. Crit Care Med 1995; 23(3):436-49.

280. Daly JM, Lieberman MD, Goldfine J, et al. Enteral nutrition with supplemental arginine, RNA, and omega-3 fatty acids in patients after operation: immunologic, metabolic, and clinical outcome [see comments]. Surgery 1992; 112(1):56-67.

281. Moore FA, Moore EE, Kudsk KA, et al. Clinical benefits of an immune-enhancing diet for early postinjury enteral feeding. J Trauma 1994; 37(4):607-15.

282. Saffle JR, Wiebke G, Jennings K, et al. Randomized trial of immune-enhancing enteral nutrition in burn patients. J Trauma 1997; 42(5):793-800; discussion 800-2.

283. Moore FA, Moore EE, Jones TN, et al. TEN versus TPN following major abdominal trauma--reduced septic morbidity. J Trauma 1989; 29(7):916-22; discussion 922-3.

284. Kudsk KA, Croce MA, Fabian TC, et al. Enteral versus parenteral feeding. Effects on septic morbidity after blunt and penetrating abdominal trauma. Ann Surg 1992; 215(5):503-11; discussion 511-3.

285. Houdijk AP, Rijnsburger ER, Jansen J, et al. Randomised trial of glutamine-enriched enteral nutrition on infectious morbidity in patients with multiple trauma [see comments]. Lancet 1998; 352(9130):772-6.

286. Senkal M, Mumme A, Eickhoff U, et al. Early postoperative enteral immunonutrition: clinical outcome and cost- comparison analysis in surgical patients [see comments]. Crit Care Med 1997; 25(9):1489-96.

287. Daly JM, Weintraub FN, Shou J, et al. Enteral nutrition during multimodality therapy in upper gastrointestinal cancer patients. Ann Surg 1995; 221(4):327-38.

288. Moore EE, Jones TN. Benefits of immediate jejunostomy feeding after major abdominal trauma-- a prospective, randomized study. J Trauma 1986; 26(10):874-81.

289. Moore FA, Feliciano DV, Andrassy RJ, et al. Early enteral feeding, compared with parenteral, reduces postoperative septic complications. The results of a meta-analysis. Ann Surg 1992; 216(2):172-83.

290. Heys SD, Walker LG, Smith I, Eremin O. Enteral nutritional supplementation with key nutrients in patients with critical illness and cancer: a meta-analysis of randomized controlled clinical trials. Ann Surg 1999; 229(4):467-77.

291. Beale RJ, Bryg DJ, Bihari DJ. Immunonutrition in the critically ill: a systematic review of clinical outcome. Crit Care Med 1999; 27(12):2799-805.

292. Eyer SD, Micon LT, Konstantinides FN, et al. Early enteral feeding does not attenuate metabolic response after blunt trauma. J Trauma 1993; 34(5):639-43; discussion 643-4.

293. Atkinson S, Sieffert E, Bihari D. A prospective, randomized, double-blind, controlled clinical trial of enteral immunonutrition in the critically ill. Guy's Hospital Intensive Care Group [see comments]. Crit Care Med 1998; 26(7):1164-72.

294. Braga M, Gianotti L, Vignali A, et al. Artificial nutrition after major abdominal surgery: impact of route of administration and composition of the diet [see comments]. Crit Care Med 1998; 26(1):24-30.

295. Juvonen PO, Tenhunen JJ, Heino AA, et al. Splanchnic tissue perfusion in acute experimental pancreatitis. Scand J Gastroenterol 1999; 34(3):308-14.

296. Berry AR, Millar AM, Taylor TV. Pancreatic blood flow in experimental acute pancreatitis. Dig Dis Sci 1982; 27(5):444-8.

297. Wang XD, Wang Q, Andersson R, Ihse I. Alterations in intestinal function in acute pancreatitis in an experimental model. Br J Surg 1996; 83(11):1537-43.

298. Hotz HG, Foitzik T, Rohweder J, et al. Intestinal microcirculation and gut permeability in acute pancreatitis: early changes and therapeutic implications. J Gastrointest Surg 1998; 2(6):518-25.

299. Li YF, Newton TJ, Weisbrodt NW, Moody FG. Intestinal migrating myoelectric complexes in rats with acute pancreatitis and bile duct ligation. J Surg Res 1993; 55(2):182-7.

300. Leveau P, Wang X, Soltesz V, et al. Alterations in intestinal motility and microflora in experimental acute pancreatitis. Int J Pancreatol 1996; 20(2):119-25.

301. Moody FG, Haley-Russell D, Muncy DM. Intestinal transit and bacterial translocation in obstructive pancreatitis. Dig Dis Sci 1995; 40(8):1798-804.

302. Runkel NS, Moody FG, Smith GS, et al. The role of the gut in the development of sepsis in acute pancreatitis. J Surg Res 1991; 51(1):18-23.

303. Ryan CM, Schmidt J, Lewandrowski K, et al. Gut macromolecular permeability in pancreatitis correlates with severity of disease in rats [see comments]. Gastroenterology 1993; 104(3):890-5.

304. Widdison AL. Pathogenesis of pancreatic infection. Ann R Coll Surg Engl 1996; 78(4):350-3.

305. Tarpila E, Nystrom PO, Franzen L, Ihse I. Bacterial translocation during acute pancreatitis in rats. Eur J Surg 1993; 159(2):109-13.

306. Gianotti L, Munda R, Alexander JW, et al. Bacterial translocation: a potential source for infection in acute pancreatitis. Pancreas 1993; 8(5):551-8.

307. Kazantsev GB, Hecht DW, Rao R, et al. Plasmid labeling confirms bacterial translocation in pancreatitis. Am J Surg 1994; 167(1):201-6; discussion 206-7.

308. Medich DS, Lee TK, Melhem MF, et al. Pathogenesis of pancreatic sepsis. Am J Surg 1993; 165(1):46-50; discussion 51-2.

309. Arendt T, Wendt M, Olszewski M, et al. Cerulein-induced acute pancreatitis in rats--does bacterial translocation occur via a transperitoneal pathway? Pancreas 1997; 15(3):291-6.

310. Widdison AL, Karanjia ND, Reber HA. Routes of spread of pathogens into the pancreas in a feline model of acute pancreatitis. Gut 1994; 35(9):1306-10.

311. Foitzik T, Fernandez-del Castillo C, Ferraro MJ, et al. Pathogenesis and prevention of early pancreatic infection in experimental acute necrotizing pancreatitis. Ann Surg 1995; 222(2):179-85.

312. Gianotti L, Munda R, Gennari R, et al. Effect of different regimens of gut decontamination on bacterial translocation and mortality in experimental acute pancreatitis. Eur J Surg 1995; 161(2):85-92.

313. Sahin M, Yol S, Ciftci E, et al. Does large-bowel enema reduce septic complications in acute pancreatitis? Am J Surg 1998; 176(4):331-4.

314. Ruokonen E, Uusaro A, Alhava E, Takala J. The effect of dobutamine infusion on splanchnic blood flow and oxygen transport in patients with acute pancreatitis [see comments]. Intensive Care Med 1997; 23(7):732-7.

315. Juvonen PO, Alhava EM, Takala JA. Gastric tonometry in assessing splanchnic tissue perfusion in acute pancreatitis. Scand J Gastroenterol 2000; 35(3):318-21.

316. Bonham MJ, Abu-Zidan FM, Simovic MO, Windsor JA. Gastric intramucosal pH predicts death in severe acute pancreatitis. Br J Surg 1997; 84(12):1670-4.

317. Ammori BJ, Leeder PC, King RF, et al. Early increase in intestinal permeability in patients with severe acute pancreatitis: correlation with endotoxemia, organ failure, and mortality. J Gastrointest Surg 1999; 3(3):252-62.

318. Foulis AK, Murray WR, Galloway D, et al. Endotoxaemia and complement activation in acute pancreatitis in man. Gut 1982; 23(8):656-61.

319. Windsor JA, Fearon KC, Ross JA, et al. Role of serum endotoxin and antiendotoxin core antibody levels in predicting the development of multiple organ failure in acute pancreatitis. Br J Surg 1993; 80(8):1042-6.

320. Luiten EJ, Hop WC, Lange JF, Bruining HA. Controlled clinical trial of selective decontamination for the treatment of severe acute pancreatitis. Ann Surg 1995; 222(1):57-65.

321. Blamey SL, Imrie CW, O'Neill J, et al. Prognostic factors in acute pancreatitis. Gut 1984; 25(12):1340-6.

322. Balthazar EJ, Robinson DL, Megibow AJ, Ranson JH. Acute pancreatitis: value of CT in establishing prognosis. Radiology 1990; 174(2):331-6.

323. Luiten EJ, Hop WC, Endtz HP, Bruining HA. Prognostic importance of gram-negative intestinal colonization preceding pancreatic infection in severe acute pancreatitis. Results of a controlled clinical trial of selective decontamination. Intensive Care Med 1998; 24(5):438-45.

324. Kotani J, Usami M, Nomura H, et al. Enteral nutrition prevents bacterial translocation but does not improve survival during acute pancreatitis [published erratum appears in Arch Surg 1999 Jun;134(6):643]. Arch Surg 1999; 134(3):287-92.

325. McClave SA, Greene LM, Snider HL, et al. Comparison of the safety of early enteral vs parenteral nutrition in mild acute pancreatitis. JPEN J Parenter Enteral Nutr 1997; 21(1):14-20.

326. Windsor AC, Kanwar S, Li AG, et al. Compared with parenteral nutrition, enteral feeding attenuates the acute phase response and improves disease severity in acute pancreatitis. Gut 1998; 42(3):431-5.

327. Kalfarentzos F, Kehagias J, Mead N, et al. Enteral nutrition is superior to parenteral nutrition in severe acute pancreatitis: results of a randomized prospective trial. Br J Surg 1997; 84(12):1665-9.

328. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992; 20(6):864-74.

329. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med 1985; 13(10):818-29.

330. Fijnheer R, Frijns CJ, Korteweg J, et al. The origin of P-selectin as a circulating plasma protein. Thromb Haemost 1997; 77(6):1081-5.

331. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16(3):1215.

332. Kremer JP, Jarrar D, Steckholzer U, Ertel W. Interleukin-1, -6 and tumor necrosis factor-alpha release is down- regulated in whole blood from septic patients. Acta Haematol 1996; 95(3-4):268-73.

333. De Groote D, Zangerle PF, Gevaert Y, et al. Direct stimulation of cytokines (IL-1 beta, TNF-alpha, IL-6, IL-2, IFN- gamma and GM-CSF) in whole blood. I. Comparison with isolated PBMC stimulation. Cytokine 1992; 4(3):239-48.

334. van der Linden MW, Huizinga TW, Stoeken DJ, et al. Determination of tumour necrosis factor-alpha and interleukin-10 production in a whole blood stimulation system: assessment of laboratory error and individual variation. J Immunol Methods 1998; 218(1-2):63-71.

335. Schroder T, Kivisaari L, Somer K, et al. Significance of extrapancreatic findings in computed tomography (CT) of acute pancreatitis. Eur J Radiol 1985; 5(4):273-5.

336. Marshall JC, Cook DJ, Christou NV, et al. Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. Crit Care Med 1995; 23(10):1638-52.

337. Powell JJ, Miles R, Siriwardena AK. Antibiotic prophylaxis in the initial management of severe acute pancreatitis. Br J Surg 1998; 85(5):582-7.

338. Folsch UR, Nitsche R, Ludtke R, et al. Early ERCP and papillotomy compared with conservative treatment for acute biliary pancreatitis. The German Study Group on Acute Biliary Pancreatitis. N Engl J Med 1997; 336(4):237-42.

339. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985; 39(Suppl 1):5-41.

340. Boehme MW, Waldherr R, Kist A, et al. Kinetics of soluble TNF-receptors and soluble adhesion molecules ICAM- 1, E-selectin and VCAM-1 under systemic rhTNF alpha therapy. Eur J Clin Invest 1996; 26(5):404-10.

341. Kuhns DB, Alvord WG, Gallin JI. Increased circulating cytokines, cytokine antagonists, and E-selectin after intravenous administration of endotoxin in humans. J Infect Dis 1995; 171(1):145-52.

342. Kingsnorth A. Role of cytokines and their inhibitors in acute pancreatitis. Gut 1997; 40(1):1-4.

343. Cummings CJ, Sessler CN, Beall LD, et al. Soluble E-selectin levels in sepsis and critical illness. Correlation with infection and hemodynamic dysfunction. Am J Respir Crit Care Med 1997; 156(2 Pt 1):431-7.

344. Cowley HC, Heney D, Gearing AJ, et al. Increased circulating adhesion molecule concentrations in patients with the systemic inflammatory response syndrome: a prospective cohort study. Crit Care Med 1994; 22(4):651-7.

345. Kayal S, Jais JP, Aguini N, et al. Elevated circulating E-selectin, intercellular adhesion molecule 1, and von Willebrand factor in patients with severe infection. Am J Respir Crit Care Med 1998; 157(3 Pt 1):776-84.

346. Inagaki T, Hoshino M, Hayakawa T, et al. Interleukin-6 is a useful marker for early prediction of the severity of acute pancreatitis. Pancreas 1997; 14(1):1-8.

347. Hynninen M, Valtonen M, Markkanen H, et al. Interleukin 1 receptor antagonist and E-selectin concentrations: a comparison in patients with severe acute pancreatitis and severe sepsis. J Crit Care 1999; 14(2):63-8.

348. Chaloner C, Blann A, Braganza JM. Endothelial cell harbingers of adult respiratory distress syndrome in acute pancreatitis [letter; comment]. Lancet 1994; 344(8930):1167.

349. Stenberg PE, McEver RP, Shuman MA, et al. A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. J Cell Biol 1985; 101(3):880-6.

350. Wagner DD. The Weibel-Palade body: the storage granule for von Willebrand factor and P-selectin. Thromb Haemost 1993; 70(1):105-10.

351. Ishiwata N, Takio K, Katayama M, et al. Alternatively spliced isoform of P-selectin is present in vivo as a soluble molecule. J Biol Chem 1994; 269(38):23708-15.

352. Jilma B, Eichler HG, Vondrovec B, et al. Effects of desmopressin on circulating P-selectin. Br J Haematol 1996; 93(2):432-6.

353. Murphy D, Imrie CW, Davidson JF. Haematological abnormalities in acute pancreatitis. A prospective study. Postgrad Med J 1977; 53(620):310-4.

354. Ranson JH, Lackner H, Berman IR, Schinella R. The relationship of coagulation factors to clinical complications of acute pancreatitis. Surgery 1977; 81(5):502-11.

355. Lasson A, Ohlsson K. Consumptive coagulopathy, fibrinolysis and protease-antiprotease interactions during acute human pancreatitis. Thromb Res 1986; 41(2):167-83.

356. Mammen EF. The haematological manifestations of sepsis. J Antimicrob Chemother 1998; 41 Suppl A:17-24.

357. Rodrick ML, Moss NM, Grbic JT, et al. Effects of in vivo endotoxin infusions on in vitro cellular immune responses in humans. J Clin Immunol 1992; 12(6):440-50.

358. Heresbach D, Letourneur JP, Bahon I, et al. Value of early blood Th-1 cytokine determination in predicting severity of acute pancreatitis. Scand J Gastroenterol 1998; 33(5):554-60.

359. Heaney ML, Golde DW. Soluble cytokine receptors. Blood 1996; 87(3):847-57.

360. van Dissel JT, van Langevelde P, Westendorp RG, et al. Anti-inflammatory cytokine profile and mortality in febrile patients [see comments]. Lancet 1998; 351(9107):950-3.

361. Pisters PW, Ranson JH. Nutritional support for acute pancreatitis. Surg Gynecol Obstet 1992; 175(3):275-84.

362. Gutierrez ED, Balfe DM. Fluoroscopically guided nasoenteric feeding tube placement: results of a 1-year study. Radiology 1991; 178(3):759-62.

363. Kirby DF, Delegge MH, Fleming CR. American Gastroenterological Association technical review on tube feeding for enteral nutrition. Gastroenterology 1995; 108(4):1282-301.

364. Eatock FC, Brombacher GD, Steven A, et al. Nasogastric feeding in severe acute pancreatitis may be practical and safe. Int J Pancreatol 2000; 28(1):25-31.

365. Heel KA, Kong SE, McCauley RD, et al. The effect of minimum luminal nutrition on mucosal cellularity and immunity of the gut. J Gastroenterol Hepatol 1998; 13(10):1015-9.

366. McCauley RD, Heel KA, Christiansen KJ, Hall JC. The effect of minimum luminal nutrition on bacterial translocation and atrophy of the jejunum during parenteral nutrition. J Gastroenterol Hepatol 1996; 11(1):65-70.

367. Okada Y, Klein N, van Saene HK, Pierro A. Small volumes of enteral feedings normalise immune function in infants receiving parenteral nutrition. J Pediatr Surg 1998; 33(1):16-9.

368. Wheeler PG, Menzies IS, Creamer B. Effect of hyperosmolar stimuli and coeliac disease on the permeability of the human gastrointestinal tract. Clin Sci Mol Med 1978; 54(5):495-501.

369. Laker MF, Menzies IS. Increase in human intestinal permeability following ingestion of hypertonic solutions. J Physiol 1977; 265(3):881-94.

370. Fong YM, Marano MA, Barber A, et al. Total parenteral nutrition and bowel rest modify the metabolic response to endotoxin in humans. Ann Surg 1989; 210(4):449-56; discussion 456-7.

371. Welsh FK, Farmery SM, MacLennan K, et al. Gut barrier function in malnourished patients. Gut 1998; 42(3):396-401.

372. Ranson JH, Rifkind KM, Roses DF, et al. Prognostic signs and the role of operative management in acute pancreatitis. Surg Gynecol Obstet 1974; 139(1):69-81.